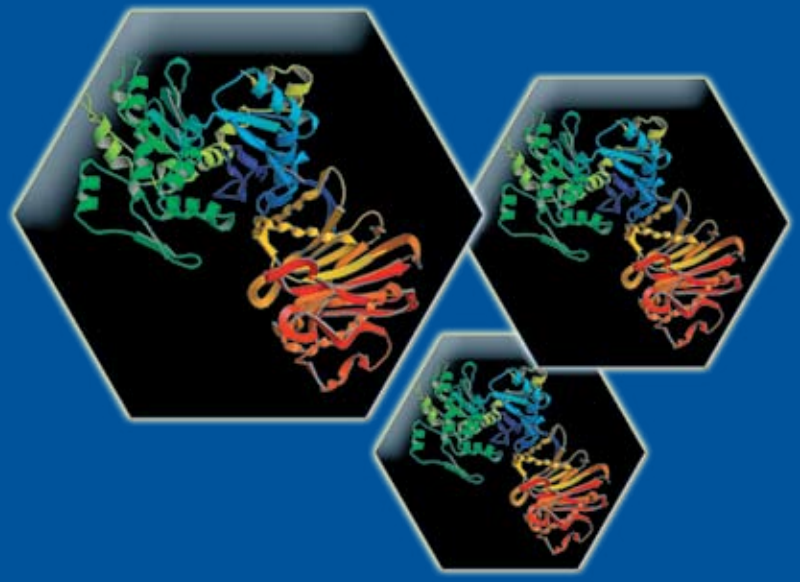
Worthington®
Biochemical Corporation

Cell Isolation Optimizing System



Kit contains an assortment of the enzymes most frequenty used for tissue dissociation and primary cell isolation applications and a Cell Isolation Guide which provides information on theory, techniques and lists hundreds of references according to tissue and species.

Store at 2 - 8°C

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Introduction

Tissue dissociation/primary cell isolation and cell harvesting are principal applications for enzymes in tissue culture research and cell biology studies. Despite the widespread use of enzymes for these applications over the years, their mechanisms of action in dissociation and harvesting are not well understood. As a result, the choice of one technique over another is often arbitrary and based more on past experience than on an understanding of why the method works and what modifications could lead to even better results.

The goal of a cell isolation procedure is to maximize the yield of functionally viable, dissociated cells, and there are nine primary parameters which affect the outcome of any particular procedure:

1. Type of tissue
2. Species of origin
3. Age of the animal
4. Dissociation medium used
5. Enzyme(s) used
6. Impurities in any crude enzyme preparation used
7. Concentration(s) of enzyme(s) used
8. Temperature
9. Incubation times

The first three items generally are not a matter of choice. To achieve suitable results the other variable conditions are best defined empirically.

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Researchers searching the scientific literature for information on the ideal enzymes and optimal conditions for tissue dissociation are often confronted with conflicting data. Much of the variation stems from the complex and dynamic nature of the extracellular matrix and from the historical use of relatively crude, undefined enzyme preparations for cell isolation applications. The extracellular matrix is composed of a wide variety of proteins, glycoproteins, lipids and glycolipids, all of which can differ in abundance from species to species, tissue to tissue and with developmental age. Furthermore, commonly used crude enzyme preparations such as Pronase, NF 1:250 and collagenase contain several proteases in variable concentrations, as well as a variety of polysaccharidases, nucleases and lipases.

Kit Contents

Enzyme	Code*	Qty/Vial	Page #
Collagenase Type 1	CLS1	500 mg dw	3
Collagenase Type 2	CLS2	500 mg dw	3
Collagenase Type 3	CLS3	500 mg dw	3
Collagenase Type 4	CLS4	500 mg dw	3
Trypsin	TRL	500 mg dw	4
Hyaluronidase	HSE	50,000 Units	4
Elastase	ESL	100 mg P	4
Papain	PAPL	100 mg P	4
Deoxyribonuclease I	DP	25 mg dw	5
Neutral Protease (Dispase®)	NPRO	10 mg dw	5
Trypsin Inhibitor	SIC	100 mg dw	5

dw = dry weight
P = protein
* The code which appears in the table for each of the enzymes corresponds to the codes found in our regular price list.

This guide summarizes our knowledge of how these enzymes accomplish the "routine" operations of tissue dissociation and cell harvesting, describes standard lab procedures; offers a logical experimental approach for establishing a cell isolation protocol, and lists many tissue-specific references.

Note: We have not limited the references listed to only those papers using Worthington enzymes. Generally speaking, the enzymes supplied in this kit can be used interchangeably for most preparations cited.

Cell Isolation Theory

Tissue Types

This section summarizes the general characteristics of extracellular matrices associated with various types of tissue. Coupled with the descriptions of individual enzymes offered in the next section, this information will aid in choosing the enzyme(s) best suited for a particular tissue.

Epithelial Tissue

In the adult, epithelium forms tissues such as the epidermis, the glandular appendages of skin, the outer layer of the cornea, the lining of the alimentary and reproductive tracts, peritoneal and serous cavities, and blood and lymph vessels (where it is usually referred to as "endothelium"). Structures derived from outpouchings from the primitive gut, including portions of the liver, pancreas, pituitary, gastric and intestinal glands are also composed of epithelial tissue.

Epithelial cells are typically packed so closely together that there is very little intercellular material between them. An extremely tight bond exists between adjacent cells making dissociation of epithelium a difficult process.

On the lateral surfaces of adjacent epithelial cells there are four distinct types of intercellular bonds: the zonula occludens, zonula adherens, macula adherens and nexus. The former three are often closely associated to form a junctional complex. In the zonula occludens, or "tight junction", there are multiple sites of actual fusion of the adjacent unit membranes interspersed by short regions of unit membrane separation of approximately 100-150 Å. In a zonula adherens, or "intermediate junction", a fine network of cytoplasmic filaments radiates from the cell membrane into the cytoplasm. The space between unit membranes of adjacent cells is approximately 150-200 Å and is composed of an intercellular amorphous substance of unknown composition. In the macula adherens, or "desmosome", there is a somewhat similar array of intracellular filaments. The adjacent unit membrane space is approximately 150-200 Å and consists of an extracellular protein and glycoprotein ground substance, often with an electron-dense bar visible within it. The integrity of the desmosome requires calcium, and it is broken down by EDTA and calcium-free media. The enzymes collagenase, trypsin and hyaluronidase can also dissociate the desmosome. The nexus, or "gap junction", covers most of the epithelial cell surface. In these areas, the unit membranes appear tightly attached and are separated by only 20Å. The intercellular material consists of an amorphous, darkly-staining substance.

On the basal surface of the epithelium where it overlays connective tissue, there is an extracellular bonding layer or sheet called the basal lamina. The lamina is composed of a network of fine, collagen-like reticular fibers embedded in an amorphous matrix of high and low molecular weight glycoproteins.

Connective Tissue

Connective tissue develops from mesenchymal cells and forms the dermis of skin, the capsules and stroma of several organs, the sheaths of neural and muscular cells and bundles, mucous and serous membranes, cartilage, bone, tendons, ligaments and adipose tissue.

Connective tissue is composed of cells and extracellular fibers embedded in an amorphous ground substance and is classified as loose or dense, depending upon the relative abundance of the fibers. The cells, which may be either fixed or wandering, include fibroblasts, adipocytes, histiocytes, lymphocytes, monocytes, eosinophils, neutrophils, macrophages, mast cells, and mesenchymal cells.

There are three types of fibers: collagenous, reticular, and elastic, although there is evidence that the former two may simply be different morphological forms of the same basic protein. The proportion of cells, fibers and ground substance varies greatly in different tissues and changes markedly during the course of development.

Collagen fibers are present in varying concentrations in virtually all connective tissues. Measuring 1-10 µm in thickness, they are unbranched and often wavy, and contain repeating transverse bands at regular intervals. Biochemically, native collagen is a major fibrous component of animal extracellular connective tissue; skin, tendon, blood vessels, bone, etc. In brief, collagen consists of fibrils composed of laterally aggregated polarized tropocollagen molecules (M.W. 300,000). Each rod-like tropocollagen unit consists of three helical polypeptide α-chains wound around a single axis. The strands have repetitive glycine residues at every third position and an abundance of proline and hydroxyproline. The amino acid sequence is characteristic of the tissue of origin. Tropocollagen units combine uniformly in a lateral arrangement reflecting charged and uncharged amino acids along the molecule, thus creating an axially repeating periodicity. Fibroblasts and possibly other mesenchymal cells synthesize the tropocollagen subunits and release them into the extracellular matrix where they undergo enzymatic processing and aggregation into native collagen fibers. Interchain cross-linking of hydroxyprolyl residues stabilizes the collagen complex and makes it more insoluble and resistant to hydrolytic attack by most proteases. The abundance of collagen fibers and the degree of cross-linking tend to increase with advancing age, making cell isolation more difficult.

Reticular fibers form a delicate branching network in loose connective tissue. They exhibit a regular, repeating subunit structure similar to collagen and may be a morphological variant of the typical collagen fibers described above. Reticular fibers tend to be more prevalent in tissues of younger animals.

Elastic fibers are less abundant than the collagen varieties. They are similar to reticular fibers in that they form branching networks in connective tissues. Individual fibers are usually less than 1 µm thick and exhibit no transverse periodicity. The fibers contain longitudinally-arranged bundles of microfibrils embedded in an amorphous substance called elastin. Like collagen, elastin contains high concentrations of glycine and proline, but in contrast has a high content of valine and two unusual amino acids, desmosine and isodesmosine. Fibroblasts and possibly other mesenchymal cells synthesize the elastin precursor, tropoelastin, and release it into the extracellular matrix where enzymes convert the lysine residues into the desmosines. Polymerization of elastin occurs during interchain cross-linking of the latter. In this state, elastin is very stable and also highly resistant to hydrolytic attack by most proteases.

The viscous extracellular ground substance in which connective tissue cells and fibers are embedded is a complex mixture of various glycoproteins, the most common being hyaluronic acid, chondroitin sulfate A, B, and C and keratin sulfate. Each of these glycoproteins is an unbranching polymer of two different alternating monosaccharides attached to a protein moiety. Hyaluronic acid, for example, contains acetyl glucosamine and glucuronate monomers and about 2% protein, while the chondroitin sulfates contain acetyl galactosamine and glucuronate or iduronate monomers and more than 15% protein. The relative abundance of these glycoproteins varies with the origin of the connective tissue.

Dissociating Enzymes

While many enzyme systems have been investigated by researchers performing primary cell isolations, the enzymes discussed here have been found satisfactory for a wide variety of tissues from many different species of various ages.

Collagenase

Bacterial collagenase is a crude complex containing a collagenase more accurately referred to as clostridiopeptidase A which is a protease with a specificity for the X-Gly bond in the sequence Pro-X-Gly-Pro, where X is most frequently a neutral amino acid. Such sequences are often found in collagen, but only rarely in other proteins. While many proteases can hydrolyze single-stranded, denatured collagen polypeptides, clostridiopeptidase A is unique among proteases in its ability to attack and degrade the triple-helical native collagen fibrils commonly found in connective tissue.

True collagenase may cleave simultaneously across all three chains or attack at a single strand. Mammalian collagenases split collagen in its native triple-helical conformation at a specific site yielding fragments, TC A and TC B, representing 3/4 and 1/4 lengths of the tropocollagen molecule. After fragmentation the pieces tend to uncoil into random polypeptides and are more susceptable to attack by other proteases.

Bacterial collagenases are usually extracted from host invasive strains. These enzymes differ from mammalian collagenases in that they attack many sites along the helix. Collagenases from *Clostridium histolyticum*, first prepared by Mandl, et al., have been most thoroughly studied. Commercially available collagenase has been limited primarily to that from *Cl. histolyticum*; although, other sources have recently become available. Clostridial collagenase also degrades the helical regions in native collagen preferentially at the X-Gly bond in the sequence Pro-X-Gly-Pro where X is most frequently a neutral amino acid. This bond in synthetic peptide substrates may also be split.

Purified clostridiopeptidase A alone is usually inefficient in dissociating tissues due to incomplete hydrolysis of all collagenous polypeptides and its limited activity against the high concentrations of non-collagen proteins and other macromolecules found in the extracellular matrix. The collagenase most commonly used for tissue dissociation is a crude preparation containing clostridiopeptidase A in addition to a number of other proteases, polysaccharidases and lipases. Crude collagenase is well suited for tissue dissociation since it contains the enzyme required to attack native collagen and reticular fibers in addition to the enzymes which hydrolyze the other proteins, polysaccharides and lipids in the extracelluar matrix of connective and epithelial tissues.

The first commercially available collagenase was offered by Worthington in 1959. At that time we offered one type of crude enzyme which we tested only for collagenase activity. Eventually, with the cooperation of many in the research community, four basic profiles were identified:

Type 1 containing average amounts of assayed activities (collagenase, caseinase, clostripain, and tryptic activities). It is generally recommended for epithelial, liver, lung, fat, and adrenal tissue cell preparations.

Type 2 containing greater proteolytic activities, especally clostripain activities. It is generally used for heart, bone, muscle, thyroid and cartilage.

Type 3 selected because of low proteolytic activity. It is usually used for mammary cells.

Type 4 selected because of low tryptic activity. It is commonly used for islets and other applications where receptor integrity is crucial.

Also available, Animal Origin Free collagenase is derived from cultures grown in medium completely devoid of animal based components and designed for bioprocessing applications where introduction of potential animal derived pathogens must be prevented. Levels of secondary proteases are similar to Types 1 and 2 for code CLSAFA and other grades are pending development.

Correlations between type and effectiveness with different tissues have been good, but not perfect, due in part to variable parameters of use. Nevertheless most researchers consider the tissue-typing of crude collagenase lots to be a valuable service. A detailed description of the Worthington collagenase assay can be found in the Worthington Enzyme Manual or at: www.worthington-biochem.com.

If you find one of the types of collagenases suitable for your cell isolation procedure, you may want to try Worthington's Collagenase Sampling Program. This cost-free program lets researchers pre-sample different lots of collagenase and evaluate them in their specific applications to achieve the best combination of cell yield and viability. (See page 40 of this guide for further information.)

COLLAGENASE

Code	Qty/Vial	Description	Activity
CLS1	500 mg dw	The usual balance of enymatic activities.	≥125 U/mg dw
CLS2	500 mg dw	Especially prepared to contain higher clostri-pain activity.	≥125 U/mg dw
CLS3	500 mg dw	Usually low in all proteolytic activities, but with normal collagenase activity.	≥100 U/mg dw
CLS4	500 mg dw	Selected to contain low tryptic activity, collagenase generally high, clostripain level low to normal.	≥160 U/mg dw

Animal Origin Free (AOF) grades of collagenase also now available, please inquire.

Activity definition:
[1 Unit liberates 1µ mole of L-leucine equivalents from collagen in 5 hours at 37°C, pH 7.5]

Trypsin

Trypsin is a pancreatic serine protease with a specificity for peptide bonds involving the carboxyl group of the basic amino acids, arginine and lysine. Trypsin is one of the most highly specific proteases known, although it also exhibits some esterase and amidase activity.

Purified trypsin alone is usually ineffective for tissue dissociation since it shows little selectivity for extracellular proteins. Combinations of purified trypsin and other enzymes such as elastase and/or collagenase have proven effective for dissociation.

"Trypsin" is also the name commercial suppliers have given to pancreatin, a crude mixture of proteases, polysaccharidases, nucleases and lipases extracted from porcine pancreas. NF 1:250, a commonly used "trypsin" preparation, has the potency to bring about the proteolytic digestion of 250 times its weight of casein under assay conditions specified by the National Formulary. It is important to realize that this assay procedure is not specific for trypsin, although pancreatin does contain this enzyme. Nomenclature notwithstanding, crude "trypsins" like NF 1:250 and 1:300 are widely used for dissociating tissues, perhaps because the tryptic and contaminating proteolytic and polysaccharidase activities do bring about a preferential attack of the extracellular matrix. It appears, however, that crude trypsin and crude collagenase dissociate tissues by different mechanisms, and difficulties are often encountered when using NF 1:250 preparations -- the most common being incomplete solubility, lot-to-lot variability, cell toxicity, and cell surface protein/receptor damage.

In tissue culture laboratories, researchers use purified trypsin to release cells into suspension from monolayers growing on the interior surfaces of culture vessels. Most cells originating from normal tissues and not highly adapted to artificial culture conditions grow in monolayers, i.e., a layer of cells one cell thick adhering to the interior surface of the culture vessel. Because such cells are more like cells in normal tissues, many tissue culture researchers are studying cells that grow in monolayer culture.

Monolayer cultures are commonly grown in glass or polystyrene roller bottles, culture flasks, or Petri dishes. Plastic vessels used in tissue culture work are specially treated to ensure good adherence of cells to the vessel walls. For a detailed discussion of cell harvesting, see page 6 of this guide.

Some of the most frequently used grades of purified trypsin for cell isolation procedures are the Worthington product Codes: TRL, TRLS, and TRTVMF. These products are suitable for cell harvesting as well as tissue dissociation.

TRYPSIN

Code	Qty/Vial	Description	Activity
TRL	500 mg dw	Supplied as a chromatographically purified, diafiltered and lyophilized powder.	≥180 U/mg P

Activity definition:
[1 Unit liberates 1µ mole of p-toluene-sulfonyl-L-arginine methyl ester (TAME) per minute at 25°C, pH 8.2, in the presence of 0.01 M calcium ion. 1 TAME unit=57.5 BAEE units=19.2 USP/NF units.]

Elastase

Pancreatic elastase is a serine protease with a specificity for peptide bonds adjacent to neutral amino acids. It also exhibits esterase and amidase activity. While elastase will hydrolyze a wide variety of protein substrates, it is unique among proteases in its ability to hydrolyze native elastin, a substrate not attacked by trypsin, chymotrypsin or pepsin. It is produced in the pancreas as an inactive zymogen, proelastase, and activated in the duodenum by trypsin. Elastase is also found in blood components and bacteria.

Because elastin is found in highest concentrations in the elastic fibers of connective tissues, elastase is frequently used to dissociate tissues which contain extensive intercellular fiber networks. For this purpose, it is usually used with other enzymes such as collagenase, trypsin, and chymotrypsin. Elastase is the enzyme of choice for the isolation of Type II cells from the lung.

ELASTASE

Code	Qty/Vial	Description	Activity
ESL	100 mg P	A lyophilized powder prepared from 2X crystallized suspension.	≥3 U/mg P

Activity definition:
[1 Unit converts 1µ mole of N-succinyl-trialynyl-p-nitroanilide per minute at 25°C, pH 8.0.]

Hyaluronidase

Hyaluronidase is a polysaccharidase with a specificity for endo-N-acetylhexosaminic bonds between 2-acetoamido-2-deoxy-beta-D-glucose and D-glucuronate. These bonds are common in hyaluronic acid and chondroitin sulfate A and C. Because these substances are found in high concentrations in the ground substance of virtually all connective tissues, hyaluronidase is often used for the dissociation of tissues, usually in combination with a crude protease such as collagenase.

HYALURONIDASE

Code	Qty/Vial	Description	Activity
HSE	50 KU	A partially purified, dialyzed, lyophilized powder.	≥300 U/mgdw

Activity definition:
A partially purified, dialyzed, lyophilized powder.

Papain

Papain is a sulfhydryl protease from *Carica papaya* latex. Papain has wide specificity and it will degrade most protein substrates more extensively than the pancreatic proteases. It also exhibits esterase activity.

With some tissues papain has proven less damaging and more effective than other proteases. Huettner and Baughman (J. Neuroscience, 6, 3044 (1986)) describe a method using papain to obtain high yields of viable, morphologically intact cortical neurons from postnatal rats which is the basis of the Worthington Papain Dissociation System.

PAPAIN

Code	Qty/Vial	Description	Activity
PAPL	100 mg P	A lyophilized powder prepared from 2X crystallized suspension containing sodium acetate.	activates* to ≥15 U/mg P

Activity definition:
[1 Unit hydrolyzes 1µ mole of BAEE per minute at 25° C, pH 6.2 after activation.]

*It is recommended that the enzyme be fully activated prior to use in a solution containing 1.1 mM EDTA, 0.067 mM mercaptoethanol and 5.5 mM cysteine-HCl for 30 minutes.

Deoxyribonuclease I

Often as a result of cell damage, deoxyribonucleic acid leaks into the dissociation medium increasing viscosity and causing handling and recovery problems. Purified deoxyribonuclease (DNase) is sometimes included in cell isolation procedures to digest the nucleic acids without damaging the intact cells.

DEOXYRIBONUCLEASE I

Code	Qty/Vial	Description	Activity
DP	25 mg dw	Partially purified. A lyophilized powder.*	≥2000 U/mg dw

Activity definition:
[1 Unit causes an increase in absorbance at 260 nm of 0.001 per minute per ml at 25°C when acting upon highly polymerized DNA at pH 5.0 (Kunitz Unit).]

*DNase is sensitive to shear denaturation — Mix gently.

Neutral Protease (Dispase®)

Neutral Protease (Dispase®) is a bacterial enzyme produced by *Bacillus polymyxa* that hydrolyses N-terminal peptide bonds of non-polar amino acid residues and is classified as an amino-endopeptidase. Its mild proteolytic action makes the enzyme especially useful for the isolation of primary and secondary (subcultivation) cell culture since it maintains cell membrane integrity.

Neutral Protease (Dispase®) is also frequently used as a secondary enzyme in conjunction with collagenase and/or other proteases in many primary cell isolation and tissue dissociation applications. Neutral Protease (Dispase®) dissociates fibroblast-like cells more efficiently than epithelial-like cells so it has also been used for differential isolation and culture applications. Other advantages are its non-mammalian (bacterial) source and its ability to be inhibited by EDTA.

NEUTRAL PROTEASE (DISPASE®)

Code	Qty/Vial	Description	Activity
NPRO	10 mg dw	Chromatigraphically purified lyophilized powder.	≥4 U/mg dw

Activity definition:
[1 Unit releases Folin-positive amino acids equivalent to 1µmole tyrosine per minute from casein at 37°C, pH 7.8]

Trypsin Inhibitor (Soybean)

The trypsin inhibitor from soybean inactivates trypsin on an equimolar basis; however it exhibits no effects on the esterolytic, proteolytic or elastolytic activities of porcine elastase. Cell isolation procedures occasionally call for a trypsin inhibitor, usually the inhibitor from soybean (Worthington Code: SIC).

TRYPSIN INHIBITOR (SOYBEAN)

Code	Qty/Vial	Description	Activity
SIC	100 mg dw	An acetone powder prepared from soybean extract.	1 mg inhibits ≥0.75 mg trypsin Code: TRL

Activity definition:
[0.75 mg TRL ≈ 135 TAME units ≈ 7760 BAEE units ≈ 2590 USP/NF units]

Cell Isolation Techniques

Methods and Materials

Working With Enzymes

- All of the enzymes Worthington offers for tissue dissociation applications are available as lyophilized powders for convenience, versatility, and stability. As such they may be stored at 2 – 8°C, and they can be shipped without special handling. While lyophilization makes shipping and storing the enzymes easier, special care is required when opening any of the vials.
- All enzymes, upon reconstitution, can be sterile filtered through a 0.22µm pore size membrane.
- Generally most of the enzymes used in cell isolation procedures (except trypsin) can be directly dissolved in a balanced salt solution or buffer of choice. Stock solutions of trypsin should be made initially by reconstituting the enzyme in 0.001N HCl. This solution can be diluted into the digestion medium or buffer immediately prior to use.
- Lyophilized proteins tend to be very hydroscopic so they should not be opened in humid areas. Be sure that any vial has been brought to room temperature before opening. Ideally, the vials should be taken from the refrigerator at least a half hour before opening, and they should be left in a dessicator. Before opening any of the vials, be sure it is not at all cool to the touch. All of the cell isolation enzymes cited in this section can be repeatedly warmed to room temperature and then returned to the refrigerator as long as these precautions are followed.
- Once diluted with media or buffer, proteolytic enzymes may undergo autolysis. Dissolve enzymes immediately before use.
- Special care must be taken with the deoxyribonuclease. This product is very prone to shear denaturation. Mix gently.
- Reconstituted enzymes should not be stored at 2–8°C. If necessary they can be aliquoted and frozen at –20°C. Avoid repeated freeze-thaw cycles.

Basic Primary Cell Isolation Protocol

(Refer to references for application specific parameters)

- For non-perfusion, mince or cut the isolated piece of tissue into 2-4 millimeter pieces with sterile scissors or scalpel.
- Add the tissue pieces to the appropriate buffer or balanced salt solution on ice and wash 2-3 times.
- Add appropriate amount of enzyme(s) and incubate at optimum temperature (usually 37°C) for appropriate time, mixing intermittently.
- Gently disperse the cells by pipeting (trituration).
- Filter the cell suspension through fine mesh.
- Allow the cells to settle and decant excess liquid containing enzymes. Wash and repeat 2-3 times.
- Resuspend cells in appropriate medium or buffer.
- Quantitate cell yield and viability.
- Seed cells for culture, if required.

Perfusion procedures require special equipment and techniques for recirculating the buffers, media and enzymes. Please refer to referenced texts for additional information and guidance.

A compilation of standard balanced salt solutions follows for reference.
A review of the reference section can be helpful in selecting an appropriate dissociation solution.

Standard Solution Table Composition of Selected Balanced Salt Solutions[a,b]

	Ringer[c]	Tyrode[de]	Gey[f]	Earle[g]	Puck[h]	Hanks[i]	Dulbecco (PBS)[jk]
NaCl	9.00	8.00	7.00	6.80	8.00	8.00	8.00
KCl	0.42	0.20	0.37	0.40	0.40	0.40	0.20
$CaCl_2$	0.25	0.20	0.17	0.20	0.012	0.14	0.40
$MgCl_2$-$6H_2O$	-	0.10	0.21	-	-	0.10	0.10
$MgSO_4$-$7H_2O$	-	-	0.07	0.10	0.154	0.10	-
Na_2HPO_4-1_2H_2O	-		3.00	-	0.39	0.12	2.31
NaH_2PO4-H_2O	-	0.05	-	0.125	-	-	-
KH_2PO_4	-	-	0.03		0.15	0.06	0.20
$NaHCO_3$	-	1.00	2.27	2.20	-	0.35	-
Glucose	-	1.00	1.00	1.00	1.10	1.00	-
Phenol Red	-	-	-	0.05	0.005	0.02	-
Atmosphere	air	air	95% air/5% CO_2	95% air/5% CO_2	air	air	air

a Amounts are given as grams per liter of solution
b In some instances the values given represent calculations from data presented by the authors to account for the use of hydrated or anhydrous salts
c S.Ringer, *J. Physiol. (London) 18*, 425 (1895)
d M.V. Tyrode, *Arch. Int. Pharmacodyn. Ther., 20*, 2025 (1910)
e R.C. Parker, "Methods of Tissue Culture", 3rd ed., p. 57, Harper, New York, 1961
f G.O. Gey and M.K. Hey, *Am J. Cancer, 27*, 55 (1936)
g W.R. Earle, *J. Natl. Cancer Inst, 4*, 165 (1943)
h T.T. Puck, S.J. Cieciura, and A. Robinson, *J. Exp. Med. 108*, 945 (1958)
i J.H. Hanks and R.E. Wallace, *Proc. Soc. Exp. Biol. Med., 71*, 196 (1949)
j PBS, phosphate-buffered saline
k R. Dulbecco and M. Vogt, *J. Exp. Med., 99*, 167 (1954)

Equilibration with 95%O_2:5%CO_2

In many cell isolation procedures it is important to the survival of the tissue during dissociation that the incubation medium be both well oxygenated and buffered at physiological pH. Both requirements are satisfied when the medium is equilibrated with 95%O_2:5%CO_2. Several balanced salt solutions contain the pH sensitive indicator dye, phenol red. When it is red or purple in color, the medium is too alkaline. This sometimes occurs when the tissue is placed in the dissociation enzyme solution. Reequilibration with O_2:CO_2 is usually necessary prior to incubation.

Gas should not be bubbled directly into any solution containing protein. This can result in frothing and denaturation of the protein with loss of biological activity. Gas can be sterilized by passage through a 0.22 micron membrane filter or through a sterile fiber plug such as the cotton plug in a sterile Pasteur or volumetric pipette. While mixing the solution, pass O_2:CO_2 continuously through the space above the liquid until color indicates pH 7.2-7.4. The balanced salt solution is often pre-gassed but should be equilibrated with sterile O_2:CO_2 each time the bottle is opened.

Buffered balanced salt solutions will usually maintain constant pH regardless of the degree of oxygenation/carbonation and as a result can be easier to work with. Certain cell types may be sensitive to particular buffer salts. The reference tables can be useful in selecting an appropriate balanced salt solution, buffer, or dissociation media for a specific application.

Trituration

(Cell dispersion through mild pumping action)

This can be a crucial procedure. It serves to break up the tissue fragments following incubation in the dissociation mix. If done too vigorously, cells will be destroyed lowering viability; too weakly and tissue fragments will be left intact lowering yield. Gentle trituration, using a 10ml pipette, constitutes filling and emptying the barrel at a rate of about 3.0ml per sec. You can best determine a suitable rate for your tissue through trial and error. Avoid bubbling the cell suspension.

Enzymatic Cell Harvesting

Most non-malignant cells growing in vitro move about and divide until they form a monolayer one cell thick completely covering the surfaces of the culture vessel. Movement and proliferation normally cease when confluence is reached. Harvesting cells for study, processing or subculture requires dissociation and detachment of the monolayer.

Limited treatment of the cell layer with the enzyme trypsin is the method most frequently applied.

It was formerly thought that trypsin preparations simply hydrolyzed a proteinaceous adhesive bonding substance responsible for the tenacious attachment of cells to their substratum with the resultant detachment of the cells from the culture vessel. It is now felt that the mechanism of action of trypsin in cell harvesting is more complex. This section summarizes recent information on this subject.

Cell Adhesion and Harvesting

During interphase, fibroblast-like cells in culture are spread out on the substratum in a characteristic, spindle-shaped configuration. There are differences of opinion as to whether the actual areas of cell adhesion are distributed over most of the undersurface of the cell or are localized in relatively narrow patches near the cell margins, principally in the vicinity of ruffling activity. In either case, these areas of adhesion appear to be composed of clusters of attachment points, each about 1 µm in diameter. The individual attachment points are apparently the distal portions of a cell cytoskeleton structure bound to the substratum.

Within minutes after subjecting cultured cells to cold temperatures, chelating agents or trypsin solutions, they change shape drastically by rounding up and blebbing. Electron micrographs show many long retraction fibers with a diameter of 0.25 – 0.5µm running from the surface of the rounded cell body to enlarged, terminal bulb attachment points previously located on the flattened cell's undersurface.

The cells remain attached to the substratum until the fibers are broken, either mechanically by tapping or shaking the culture vessel, or chemically by the continued action of chelators and/or trypsin. (Cold temperatures alone are sufficient for rounding up but not for detachment. These conditions also greatly diminish the entry of trypsin into the cell.) Soon after cell detachment from the surface of the culture vessel, and subculture into new vessels containing trypsin-free medium, cytoplasm flows into the broken retraction fibers and refills them. Within an hour the rounded cells begin to take on their characteristic shape.

Trypsin for Cell Harvesting

In 1916, Rous and Jones used "the trypsin powders of Merck, Brubler and Kahlbaum" to digest the plasma clots in which living cells were growing in order to obtain a cell suspension for subculturing. Vogelaar and Erlichman in 1934 were the next researchers to utilize the digestive enzymes in a crude trypsin preparation to liquify the coagulated plasma in which human fibroblasts were growing prior to subculturing. Techniques using trypsin similar to those used today were introduced by Scherer, Syverton and Gey in 1953 to harvest the then newly cultivated HeLa cell strain for subculturing and biochemical analysis. These workers tested both recrystallized trypsin and NF 1:250 trypsin for cell harvesting and found that the purified trypsin was more potent and less toxic to cells. Nevertheless the NF 1:250 preparation was employed for routine harvesting simply because it was less expensive.

Relatively crude pancreatic preparations like NF 1:250 trypsin are still used today for cell harvesting in spite of the fact that they exhibit considerable lot-to-lot variability and contain extraneous substances and other enzymatic activities. Impurities in crude trypsin can cause unnecessary damage to cells and a reduction of cloning efficiency. Use of higher purity crystalline trypsin can eliminate many of these difficulties.

None of the contaminants present in the NF 1:250 materials appears to be essential for cell harvesting activity since purified trypsin is very effective for monolayer dissociation, and since crude NF 1:250 trypsin plus soybean trypsin inhibitor is ineffective.

McKeehan and Ham report markedly improved viability and multiplication potential to single cells in low serum medium when harvesting with crystalline trypsin at reduced temperatures, i.e., at 4°C.

Cell Release Procedure

In order to transfer or pass cells in monolayer culture from one culture vessel to another it is necessary to release cells from the monolayer into suspension so that they can be easily handled by pipetting and diluting.

Releasing cells from the monolayer is almost always accomplished with purified trypsin by a procedure known as trypsinization.
A usual trypsinization procedure follows.

Trypsinization Procedure

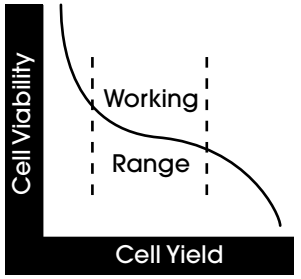
1. Remove culture medium from cells.
2. Add sterile trypsin solution (in BSS-balanced salt solution, normally calcium-free Hanks).
3. Allow trypsin solution to act on monolayer for several minutes at room temperature or 37°C. (or longer at 2-8°C.)
4. Remove trypsin solution gently, so as not to disturb cells.
5. Add BSS or media (often with serum or trypsin inhibitor to inactivate residual trypsin) and agitate vessel to disrupt monolayer and suspend cells.

Optimization Techniques

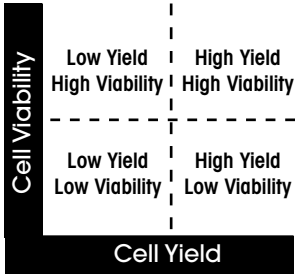
General Guidelines

Although optimization of a cell isolation procedure for a particular cell type is dependent upon the adequate recovery of cells having various required characteristics, some guidelines can be established. The information in this guide regarding cell isolation and the enzymes used, when combined with logic and suitable experimental design, should lead to the development of a satisfactory cell isolation method. (See Freshney, 1987 for a detailed discussion)

The complex relationship between cell yield and viability can be represented by the simplified illustrations shown below. In general there is an area of optimized recovery balanced between yield and viability; working near the middle of this range will reduce variability in the results of the cell isolation procedure. Understanding this relationship and how it can vary with a particular cell type and application, can make the optimization process easier.



For troubleshooting purposes various possible results, along with suggested corrective actions are listed below. Keep in mind that there are no clear lines between the quadrants but rather converging zones with variable areas of overlap.



Low Yield/Low Viability: *Over/under dissociation, cellular damage.* Change to less digestive type enzyme and/or decrease working concentration. (e.g. from trypsin to collagenase/ from Type 2 collagenase to Type 1).

Low Yield/High Viability: *Under dissociation.* Increase enzyme concentration and/or incubation time and monitor both yield and viability response.

If yield remains poor, evaluate a more digestive type enzyme and/or the addition of secondary enzyme(s).

High Yield/Low Viability: *Good dissociation, cellular damage.* Enzyme overly digestive and/or at too high a working concentration. Reduce concentration and/or incubation time and monitor yield and viability response.

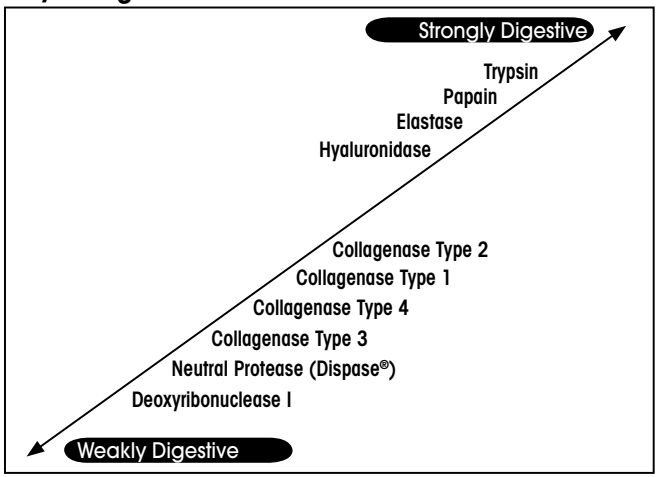
Try diluting the proteolytic action by adding bovine serum albumin (BSA) (0.1 = 0.5% w/v) or soybean trypsin inhibitor (0.01 - 0.1% w/v) to the dissociation.

Try using less proteolytic enzyme although yield may be affected and should be monitored.

High Yield/High Viability: *The place to be!* Consider evaluating the effect of dissociation parameters to learn their limitations for future reference.

A scale showing the relative digestive power of the enzymes commonly used follows for reference. Refer to this scale when troubleshooting a dissociation and planning isolation strategy.

Enzyme Digestion Scale



Optimization Strategy

Review the reference tables starting on page 11 for the particular tissue and cell type of interest, and then apply this information to the practical application of tissue dissociation. An example of a basic optimization strategy follows:

Based upon the enzyme(s) cited, working concentrations and the buffer or media system used, set up proposed preliminary dissociation conditions similar to the closest available reference(s) listed in the tables.

Note: If a majority of the most similar referenced procedures cite the use of more than one enzyme, optimize the concentration of the primary enzyme (the one at the highest relative concentration) before adding the secondary enzyme(s). For example, if the two most similar references cite collagenase 0.1% with DNase 0.01% and collagenase 0.075% with hyaluronidase 0.025%, optimize the collagenase concentration empirically before evaluating the effects of either the hyaluronidase or the deoxyribonuclease. After optimizing the primary enzyme's concentration and incubation conditions evaluate any secondary enzyme(s).

1. Initially vary the concentration of the primary enzyme approximately 50% relative to the referenced procedure(s). The above example of collagenase concentrations 0.1% and 0.075% suggests an evaluation of enzyme concentrations between 0.025% and 0.15%. The concentration increments should be evenly distributed to cover this entire range. As a result incremental concentrations of 0.025%, 0.05%,0.075%, 0.10%, 0.125% and 0.15% would be indicated. To simplify the initial screening the middle of the range can be selected and, after evaluation of yield and viability results, a decision can be made regarding the need for further studies. In this case initial collagenase concentrations evaluated may be 0.05%, 0.075%, 0.10% and 0.125%.

Note: Historically, most tissue dissociation and cell isolation protocols have cited the enzyme concentration used in terms of weight per unit volume (w/v). More recently, however, some researchers have begun to use the enzymes on an activity basis, that is, units per milliliter (u/ml). Use either method but consider the advantages and disadvantages of each:

1. The traditional weight per unit volume method most likely resulted from the use of cruder, partially purified mixtures of enzymes and is used independently of any specific or contaminating activities which may be present. With some of these crude preparations the lot-to-lot variation can be significant resulting in up to a two-fold difference in the amount of enzymatic activity added on a weight basis.
2. Adding by activity can result in a possible two-fold difference in the amount of weight added to a dissociation; however, normalizes the potency used based upon the primary activity for each lot.

Both methods ignore the relative contaminant activity levels. Upon establishing a basic method, consider pre-sampling different lots of enzyme(s) to evaluate these factors and to select a lot of enzyme which has minimal effect upon the critical parameters of a specific application.

Important: For accurate evaluation of a particular procedure's performance, cell yield and viability should be quantitated and compared. After optimizing basic dissociation and isolation conditions, the specific application parameters such as metabolic function(s) or receptor binding capability should also be evaluated. Based upon these results the method may be judged suitable for use or re-optimized for higher retention of native cellular charactaristics.

Cell Quantitation

It is important to quantitate the results of each dissociation step in order to effectively evaluate each procedure. The use of a cell counting chamber (hemocytometer) for yield quantitation and the use of trypan blue for viability quantitation are recommended. The use of a hemocytometer for cell yield quantitation is outlined; however, newcomers to this procedure can refer to more detailed discussions (see Freshney, Culture of Animal Cells, page 335).

Required Supplies:

Improved Neubauer Hemocytometer

Cell Compatible Media or BSS

Pasteur Pipet or Micropipettor

Microscope (10X)

Counter

Procedure:

1. Carefully clean the counting chamber surface and the coverslip of the hemocytometer with 70% isopropanol and allow to air dry. Be careful not to scratch these surfaces.

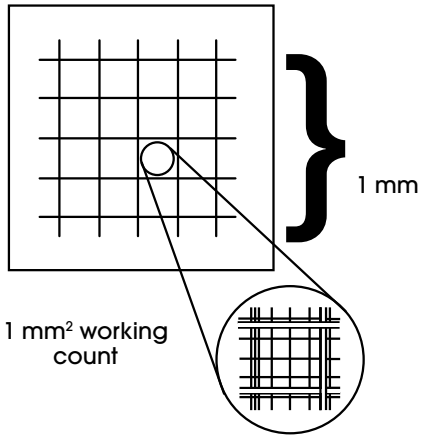
2. Wet the sides of the coverslip with reagent grade water and align the coverslip over the counting chamber.

3. Take a well mixed 20-50 µl aliquot of the dissociated cell suspension using either a Pasteur pipet or a micropipettor only drawing the cells into the tip. Immediately transfer the cell suspension to the counting chamber by placing the tip of the pipet at the edge of the chamber and allowing the chamber to fill completely via capillary action. Do not over- or underfill the chamber.

4. Repeat this procedure using another aliquot sample for the second chamber on the opposite side of the hemocytometer.

5. Place the hemocytometer on the microscope stage and, using the 10X objective, focus on the counting chamber grid lines. Adjust the contrast as needed to clearly see both the grid and the dispersed cells.

6. Adjust the field area by slowly moving the slide to obtain a central grid bounded by three lines on all sides (see figure below). Count the total number of cells present in this 1 mm^2 area including those cells which are on the top and left borders and excluding those on the right and bottom borders.



7. For accuracy count at least 100-500 cells. Depending upon yield and density more or fewer areas may be counted.

8. Repeat the count for the second chamber. If no second chamber exists, the slide should be cleaned and the process repeated.

Calculation:

$C = \tilde{N} \times 10^4$

where C = cells per milliliter
$\tilde{N}$ = average of cells counted
10^4 = volume conversion factor for 1 mm^2

Total Yield = C x V
where V = total volume of cells (ml)

Example:

$Count_1$ = 182 cells/mm^2
$Count_2$ = 175 cells/mm^2

Volume of Cells = 55 ml

$$\text{Average cells counted} = \frac{Count_1 + Count_2}{2} = \frac{182 + 175}{2} = 178.5$$

$C = 178.5 \times 10^4 = 1{,}785{,}000$ cells/ml

Total yield = C x V = 1,785,000 x 55 = 98,175,000 cells

Note: For best results the cell density should be at least 10^5 cells per milliliter. Common errors occur by improper mixing of the cell suspension prior to sampling and/or by allowing the cells to settle in the pipet prior to loading the hemocytometer counting chamber. Avoid the counting of multiple cell aggregates; the presence of aggregates indicates incomplete dissociation which may require further optimization of the isolation parameters. A single cell suspension provides the best results.

Measure of Viability

One of the simplest methods to approximate cell viability is the dye exclusion technique. This method utilizes an indicator dye to demonstrate cell membrane damage. Cells which absorb the dye become stained and are considered non-viable. Dyes such as trypan blue, erythrosin, and nigrosin are commonly used with trypan blue being the most common in preliminary cell isolation procedures.

This procedure can be performed along with the cell counting procedure but cell density may require adjustment in order to obtain approximately 10^6 cells per milliliter.

Procedure

1. Mix 1 drop of trypan blue with one drop of the cell suspension and allow 1 - 2 minutes for absorption

2. Prepare hemocytometer and load chambers as described in “Cell Quantitation”.

3. Count both the total number of cells and the number of stained (dark) cells.

Calculation

$$\text{Percent Viability} = \frac{\text{Total Cells Counted - Stained Cells}}{\text{Total Cells Counted}} \times 100$$

Example

Total Cells / 1 mm^2 = 182
Stained Cells = 24

$$\text{\% Viability} = \frac{182 - 24}{182} = \frac{158}{182} \times 100$$

= 86.8% Viability

Note: Dye exclusion viability procedures tend to give high estimates of cell viability when compared to cell attachment or metabolic assays, but for optimization of cell isolation procedures trypan blue does provide a rapid estimate of dissociation performance in conjunction with yield quantitation.

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Mandl, I., MacLennan, J.D., Howes, E.L., DeBellis, R.H., and Sohler, A.: Isolation and Characterization of Proteinase and Collagenase *from Cl. histolyticum*, *J. Clin. Invest., 32* ,*1323* (1953).

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Worthington Enzyme Manual, Worthington Biochemical Corp., Lakewood, NJ, 1993

Worthington Tissue Dissociation Guide, Worthington Biochemical Corp., Lakewood, NJ, 2012

Tissue Tables

Each of the following tables lists references related to a specific type of tissue. For your convenience the listings are arranged according to species. We have attempted to find references for a large variety of species of various ages, and from those to select the most recent papers. Worthington also publishes the Tissue Dissociation Guide which includes additional information. The Tissue Dissociation Guide is available on our websites, www.worthingtonbiochem.com or www.tissuedissociation.com. To obtain a printed copy, please contact Customer Service at 800.445.9603 from anywhere within the United States or Canada or via e-mail at: custservice@worthington-biochem.com.

Index to Tables

Note: The following abbreviations appear throughout the Cell Isolation Optimizing Insert:

BALB ..... Bagg Albine (obtained from H.J Bagg in 1923)
BSA ..... Bovine Serum Albumin
BSS ..... Balanced Salt Solution
CF ..... Calcium Free
CLSPA ..... Worthington Purified Collagenase
CMF ..... Calcium Magnesium Free
DMEM ..... Dulbecco's Modified Eagle Medium
EBSS ..... Earle's Balanced Salt Solution
FBS ..... Fetal Bovine Serum
HBSS ..... Hank's Balanced Salt Solution
HIS ..... Hepatocyte Isolation System
L-15 ..... Liebowitz L-15 Medium
MEM ..... Minimum Essential Medium
NCIS ..... Worthington Neonatal Cardiomyocyte Isolation System
PBS ..... Phosphate Buffered Saline
PDS ..... Worthington Papain Dissociation System
RPMI ..... Roswell Park Memorial Institute (Moore, et al, Tissue Culture Association Manual, 3, 503-508, 1976)
SD ..... Sprague-Dawley
SW ..... Swiss Webster

Species		Cell(s)	Enzyme(s)	Medium	Reference
Bovine	Bovine	Adipocytes	Collagenase Type 1: 40 u/ml	Krebs-Ringer bicarbonate	Yang YT, Baldwin RL: Preparation and metabolism of isolated cells from bovine adipose tissue, *J Dairy Sci 56*, 350-65, 1973 (*10340*)
Canine	Canine	Renal adipose derived cells	Collagenase Type 1: 0.3%	DMEM	Basu, J., Genheimer, C., Sangha, N., Quinlan, S., Guthrie, K., Kelley, R., Ilagan, R., Jain, D., Bertram, T. and Ludlow, J.: Organ Specific Regenerative Markers in Peri-Organ Adipose: Kidney., Lipids Health Dis Vol. 10, 171, 2011 (10665)
	Canine, 20-25 kg	Adipose stem cells	Collagenase: see reference	Media-199	Fischer, L., McIlhenny, S., Tulenko, T., Golesorkhi, N., Zhang, P., Larson, R., Lombardi, J., Shapiro, I. and DiMuzio, P.: Endothelial Differentiation of Adipose-Derived Stem Cells: Effects of Endothelial Cell Growth Supplement and Shear Force., J Surg Res 152, 157, 2009 (10599)
Equine	Equine	Adipose derived stem cells	Collagenase Type 1: 0.1%	PBS	Vidal, M., Robinson, S., Lopez, M., Paulsen, D., Borkhsenious, O., Johnson, J., Moore, R. and Gimble, J.: Comparison of Chondrogenic Potential in Equine Mesenchymal Stromal Cells Derived from Adipose Tissue and Bone Marrow., Vet Surg Vol. 37, 713, 2008 (10561)
Fish	Fish, Atlantic salmon	Preadipocytes	Collagenase Type 1: 0.1%	HBSS	Todorcevic, M., Vegusdal, A., Gjoen, T., Sundvold, H., Torstensen, B., Kjaer, M. and Ruyter, B.: Changes in Fatty Acids Metabolism During Differentiation of Atlantic Salmon Preadipocytes; Effects of n-3 and n-9 Fatty Acids., Biochim Biophys Acta 1781, 326, 2008 (10597)
Gerbil	Gerbil of unknown age (also rat, hamster, rabbit, lamb, guinea-pig)	Brown fat	Collagenase Type 1: 0.10%	Bicarbonate buffer	Nedergaard, J. and Lindberg, O.: The Brown Fat Cell, *Int Rev Cytol 74*, 187, 1982 (*544*)
Guinea-Pig	Guinea-pig, adult (also rat, hamster, gerbil, rabbit, lamb)	Brown fat	Collagenase Type 1: 0.10%	Bicarbonate buffer	Nedergaard, J. and Lindberg, O.: The Brown Fat Cell, *Int Rev Cytol 74*, 187, 1982 (*544*)
Hamster	Hamster, adult (also rat, gerbil, rabbit, lamb, guinea-pig)	Brown fat	Collagenase Type 1: 0.10%	Bicarbonate buffer	Nedergaard, J. and Lindberg, O.: The Brown Fat Cell, *Int Rev Cytol 74*, 187, 1982 (*544*)
Human	Human, male 40-60 years	Adipose derived stem cells	Collagenase: 0.25% Deoxyribonuclease I: 0.002%	PBS	Blasi, A., Martino, C., Balducci, L., Saldarelli, M., Soleti, A., Navone, S., Canzi, L., Cristini, S., Invernici, G., Parati, E. and Alessandri, G.: Dermal Fibroblasts Display Similar Phenotypic and Differentiation Capacity to Fat-Derived Mesenchymal Stem Cells, but Differ in Anti-Inflammatory and Angiogenic Potential, Vasc Cell Vol. 3, 5, 2011 (10486)
Mouse	Mouse	Stem and progenitor	Collagenase Type 2: 0.2%	HBSS	Han, J., Koh, Y., Moon, H., Ryoo, H., Cho, C., Kim, I. and Koh, G.: Adipose Tissue is an Extramedullary Reservoir for Functional Hematopoietic Stem and Progenitor Cells., Blood 115, 957, 2010 (10494)
	Mouse, 3 week	Adipocytes	Collagenase Type 1: 0.2%	HBSS	De Matteis, R., Zingaretti, M., Murano, I., Vitali, A., Frontini, A., Giannulis, I., Barbatelli, G., Marcucci, F., Bordicchia, M., Sarzani, R., Raviola, E. and Cinti, S.: In Vivo Physiological Transdifferentiation of Adult Adipose Cells., Stem Cells 27, 2761, 2009 (10552)
Porcine	Porcine, female, <1 year	Adipose mesenchymal stem	Collagenase Type 1: 0.1%	DMEM	Williams, K., Picou, A., Kish, S., Giraldo, A., Godke, R. and Bondioli, K: Isolation and Characterization of Porcine Adipose Tissue-Derived Adult Stem Cells., Cells Tissues Organs 188, 251, 2008 (10370)
Rat	Rat, Lewis, male	Renal adipose derived cells	Collagenase Type 1: 0.3%	DMEM	Basu, J., Genheimer, C., Sangha, N., Quinlan, S., Guthrie, K., Kelley, R., Ilagan, R., Jain, D., Bertram, T. and Ludlow, J.: Organ Specific Regenerative Markers in Peri-Organ Adipose: Kidney., Lipids Health Dis Vol. 10, 171, 2011 (10665)
	Rat, SD, neonatal	Brown adipocytes	Collagenase Type 4: 0.1% Neutral Protease: 0.1% Trypsin: 0.05%	PBS	Liu, Z., Wang, H., Zhang, Y., Zhou, J., Lin, Q., Wang, Y., Duan, C., Wu, K. and Wang, C.: Efficient Isolation of Cardiac Stem Cells from Brown Adipose., J Biomed Biotechnol Vol. 2010, 104296, 2010 (10598)
	Rat, Wistar, 4 week	Adipocytes	Collagenase: 0.2%	Ham's F12	Aoki, S., Toda, S., Sakemi, T., and Sugihara, H.: Coculture of Endothelial Cells and Mature Adipocytes Actively Promotes Immature Preadipocyte Development In Vitro, *Cell Struct Funct 28*, 55, 2003 (*9791*)
	Rat, SD	Adipocytes	Collagenase Type 1: 0.2%	KRHB	Mora, S., Yang, C., Ryder, J., Boeglin, D and Pessin, J: The MEF2A and MEF2D Isoforms are Differentially Regulated in Muscle and Adipose Tissue during States of Insulin Deficiency, *Endocrinology 142*, 1999, 2001 (*9796*)
	Rat, SD, male, 4-7 weeks	Brown adipocytes	Deoxyribonuclease I: 0.5%	DMEM	Omatsu-Kanbe, M., and Matsuura, H.: Inhibition of Store-operated Ca2+ Entry by Extracellular ATP in Rat Brown Adipocytes, *J Physiol 521 (3)*, 601, 1999 (*1307*)
	Rat	Adipocytes	Collagenase Type 2: 0.2%	DMEM /F-12	Serrero, G: Primary Culture in Defined Medium of Adipocyte Precursors,*Cell & Tissue Culture: Laboratory Procedures Vol. 1*,Doyle, A., Griffiths, J., and Newell, D., John Wiley and Sons, Ltd., 11B:6.1, 1995 (*1285*)
	Rat, SD, male, 130-160 g	Adipose Epididymal fat pads	Collagenase: 0.3%	Kreb's-Ringer bicarbonate buffer modified	Charron, M.J. and Kahn, B.B.: Divergent Molecular Mechanisms for Insulin-Resistant Glucose Transport in Muscle and Adipose Cells *In Vivo*, *J Biol Chem 265*, 7994, 1990 (*571*)
	Rat, 3 day	Preadipocytes	Collagenase Type 3: 0.10%	Parker Medium 199	Gaben-Cogneville, A., Poussin, B., Chamblier, M., Forgue-Fafitte, M., and Rosselin, G.: Development of Insulin and Epidermal Growth Factor Receptors During the Differentiation of Rat Preadipocytes in Primary Culture, *Biochim Biophys Acta 968*, 231, 1988 (*336*)
	Rat, SD, various weights and ages	Brown adipocytes Interscapular & cervical depots	Collagenase: 0.2% Soybean Trypsin Inhibitor: 0.3%	Krebs Ringer bicarbonate buffer	Woodward, Julie A. and Saggerson, E.: Effect of Adenosine Deaminase, N6-Phenylisopropyladenosine and Hypothroidism on the Responsiveness of Rat Brown Adipocytes to Noradrenaline, *Biochem J 238*, 395, 1986 (*311*)
	Rat, CD, male, 150-200 g	Adipocytes Epididymal fat pads	Collagenase: 0.1%	Krebs Ringer bicarbonate buffer	Pessin, J.E., Gitomer, W., Oka, Y., Oppenheimer, C.L., and Czech, M.P.: ß-Adrenergic Regulation of Insulin and Epidermal Growth Factor Receptors in Rat Adipocytes, *J Biol Chem 258*, 7386, 1983 (*558*)

[CF=Calcium Free • CMF=Calcium Magnesium Free • BSS=Balanced Salt Solution]

Species		Cell(s)	Enzyme(s)	Medium	Reference
Bovine	Bovine	Chromaffin cells	Collagenase: 0.1% Deoxyribonuclease I: 30 u/ml	DMEM	Hahm, S., Chen, Y., Vinson, C. and Eiden, L.: A Calcium-Initiated Signaling Pathway Propagated Through Calcineurin and cAMP Response Element-Binding Protein Activates Proenkephalin Gene Transcription after Depolarization., *Mol Pharmacol 64*, 1503, 2003 (*10565*)
	Bovine, 6 month	Chromaffin	Collagenase Type 1: 0.125%	Locke's solution	Moustafa T, Girod S, Tortosa F, Li R, Sol JC, Rodriguez F, Bastide R, Lazorthes Y, Sallerin B: Viability and functionality of bovine chromaffin cells encapsulated into alginate-PLL microcapsules with a liquefied inner core, *Cell Transplant 15*, 121-33, 2006 (*10341*)
	Bovine	Chromaffin	Collagenase: 0.2%	Locke's solution	Ortega, J., Sagen, J., and Pappas, G.: Short-term Immunosuppression Enhances Long-term Survival of Bovine Chromaffin Cell Xenografts in Rat CNS, *Cell Transplant 1*, 33, 1992 (*359*)
	Bovine	Chromaffin	Deoxyribonuclease I: 30 u/mg	HEPES	Zhu, J., Li, W., Toews, M., and Hexum, T.: Neuropeptide Y Inhibits Forskolin-Stimulated Adenylate Cyclase in Bovine Adrenal Chromaffin Cells *via* a Pertussis Toxin-Sensitive Process, *J Pharmacol Exp Ther 263 (3)*, 1479, 1992 (*1232*)
	Bovine (also rat)	Heart Adrenal chromaffin Paraneurons	Trypsin: 0.06%	25mM HEPES buffered Locke's solution, CMF	Trifaro, J., Tang, R., and Novas, M.: Monolayer Co-Culture of Rat Heart Cells and Bovine Adrenal Chromaffin Paraneurons, *In Vitro Cell Dev Biol 26*, 335, 1990 (*438*)
	Bovine	Chromaffin	Collagenase Type 1: 0.25 %	DMEM	Dahmer, M., Hart, P., and Perlman, R.: Studies on the Effect of Insulin-Like Growth Factor-I on Catecholamine Secretion from Chromaffin Cells, *J Neurochem 54 (3)*, 931, 1990 (*1231*)
	Bovine	Chromaffin	Collagenase: 0.05%	Locke's solution, CMF	Aunis, D., Rotllan, P., and Miras-Portugal, M.: Incorporation of Adenosine into Nucleotides of Chromaffin Cells in Culture, *Neurochem Int 7*, 89, 1985 (*644*)
	Bovine	Chromaffin	Collagenase: 0.15%	Kreb's, CMF	Almazan, G., Aunis, D., Garcia, A., Montiel, C., Nicolas, G., and Sanchez-Garcia, P.: Effects Of CLS on the Release of Noradrenaline From Chromaffin Cells, *Br J Biomed Sci 81*, 599, 1984 (*343*)
	Bovine	Chromaffin	Collagenase: 0.1%	(see reference)	Pollard, H., Pazoles, C., Creutz, C., Scott, J., Zinder, O., and Hotchkiss, A.: An Osmotic Mechanism For Exocytosis From Dissociated Chromaffin Cells, *J Biol Chem 259*, 1114, 1984 (*559*)
	Bovine	Chromaffin	Collagenase Type 1: 0.05%	CF Kreb's	Cena, V., Garcia, A., Montiel, C., and Sanchez-Garcia, P.: Uptake of [^{3}H]-nicotine and [^{3}H]-noradrenaline by Cultured Chromaffin Cells, *Br J Pharmacol 81*, 119, 1984 (*342*)
	Bovine	Chromaffin	Collagenase Type 1: 0.025%	HBSS, modified	Waymire, J., Bennett, W., Boehme, R., Hankins, L., Gilmer-Waymire, K., and Haycock, J.: Bovine Adrenal Chromaffin Cells: High-Yield Purification and Viability in Suspension Culture, *J Neurosci Methods 7*, 329, 1983 (*608*)
	Bovine	Medulla	Hyaluronidase: 0.2%	Saline w/BSA 0.5%	Knight, D. and Baker, P.: Stimulus-Secretion Coupling in Isolated Bovine Adrenal Medullary Cells, *Q J Exp Physiol 68*, 123, 1983 (*715*)
	Bovine	Medulla	Collagenase: 0.2%	Krebs-Ringer bicarbonate buffer, CMF	Greenberg, A. and Zinder, O.: alpha- and beta-Receptor Control of Catecholamine Secretion from Isolated Adrenal Medulla Cells, *Cell Tissue Res 226*, 655, 1982 (*356*)
	Bovine	Chromaffin	Deoxyribonuclease I: 15 µg/ml	Medium A (see reference)	Wilson, S.P., and Viveros, O.H.: Primary Culture of Adrenal Medullary Chromaffin Cells in a Chemically Defined Medium, *Exp Cell Res 133*, 159, 1981 (*392*)
	Bovine	Medulla	Protease: 0.2%	Saline	Baker, P., and Knight., D: Calcium Control of Exocytosis and Endocytosis in Bovine Adrenal Medullary Cells, *Phil Trans R Soc Lond 296*, 83, 1981 (*1158*)
	Bovine	Chromaffin	Collagenase: 0.05%	Locke's solution, CMF	Trifaro, J.M., and Lee, R.W.: Morphological Characteristics and Stimulus-Secretion Coupling in Bovine Adrenal Chromaffin Cell Cultures, *Neuroscience 5*, 1533, 1980 (*647*)
	Bovine, adult (also rat, Hanover-Wistar, young; guinea-pig, newborn)	Chromaffin	Collagenase: 0.5%	HBSS	Unsicker, K., Rieffert, B., and Ziegler, W.: Effects of Cell Culture Conditions, Nerve Growth Factor, Dexamethasone, and Cyclic AMP on Adrenal Chromaffin Cells *In Vitro*, *Adv Biochem Psychopharmacol 255*, 51, 1980 (*713*)
	Bovine	Chromaffin	Collagenase: 0.25%	F-12 medium	Kumakura, K., Karoum, F., Guidotti, A., and Costa, E.: Modulation of Nicotinic Receptors by Opiate Receptor Agonists in Cultured Adrenal Chromaffin Cells, *Nature 283*, 489, 1980 (*714*)
Guinea-Pig	Guinea-pig, 500-700 g	Chromaffin Medulla	Collagenase:	BSS (see reference)	Role, L.W., Leeman, S.E., and Perlman, R.L.: Somatostain and Substance P Inhibit Catecholamine Secretion from Isolated Cells of Guinea-pig Adrenal Medulla, *Neurochem Int 6*, 1813, 1981 (*643*)
	Guinea-pig (also rat, Hanover-Wistar, young; newborn; cattle)	Chromaffin	Collagenase: 0.5%	HBSS	Unsicker, K., Rieffert, B., and Ziegler, W.: Effects of Cell Culture Conditions, Nerve Growth Factor, Dexamethasone, and Cyclic AMP on Adrenal Chromaffin Cells *In Vitro*, *Adv Biochem Psychopharmacol 255*, 51, 1980 (*713*)
	Guinea-pig	Adrenal Chromaffin	Collagenase: 0.05%-0.20%	Kreb's-Ringer bicarb glucose buffer, CF	Hochman, J., and Perlman, R.L.: Catecholamine Secretion by Isolated Adrenal Cells, *Biochim Biophys Acta 421*, 168, 1976 (*320*)
Hamster	Hamster (*Mesocricetus auratus*) 100-150 g	Adrenal Chromaffin	Hyaluronidase: 0.20%	Kreb's Ringer bicarbonate buffer	Liang, B.T., and Perlman, R.L.: Catecholamine Secretion by Hamster Adrenal Cells, *J Neurochem 32*, 927, 1979 (*606*)
Human	Human	Chromaffin cells	Collagenase: 0.2%	Locke's solution	Jeon, Y., Baek, W., Chung, S., Shin, N., Kim, H., and Lee, S.: Cultured Human Chromaffin Cells Grafted in Spinal Subarachnoid Space Relieves Allodynia in a Pain Rat Model., *Korean J Anesthesiol Vol. 60*, 357, 2011 (*10566*)
	Human	Adrenocortical	Collagenase Type 1: 0.2% Deoxyribonuclease I: 0.01%	Kreb's Ringer	Caroccia, B., Fassina, A., Seccia, T., Recarti, C., Petrelli, L., Belloni, A., Pelizzo, M. and Rossi, G.: Isolation of Human Adrenocortical Aldosterone-Producing Cells by a Novel Immunomagnetic Beads Method., *Endocrinology 151*, 1375, 2010 (*10680*)
Mouse	Mouse, embryonic	Chromafin cells	Papain: 20-25 u/ml	DMEM	Tian Jin-Hua, Wu Zheng-Xing, Unzicker Michael, Lu Li, Cai Qian, Li Cuiling, Schirra Claudia, Matti Ulf, Stevens David, Deng Chuxia, Rettig Jens, Sheng Zu-Hang: The role of Snapin in neurosecretion: snapin knock-out mice exhibit impaired calcium-dependent exocytosis of large dense-core vesicles in chromaffin cells, *J Neurosci 25*, 10546-55, 2005 (*10118*)

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Adrenal (Con't)

Species		Cell(s)	Enzyme(s)	Medium	Reference
Ovine	Ovine, fetal	Adrenocortical	Collagenase Type 1: 0.4%	DMEM/Ham's F12	Valego, N., Su, Y., Carey, L., Young, S., Tatter, S., Wang, J. and Rose, J.: Hypothalamic-Pituitary Disconnection in Fetal Sheep Blocks the Peripartum Increases in Adrenal Responsiveness and Adrenal ACTH Receptor Expression., *Am J Physiol Regul Integr Comp Physiol 289*, R410, 2005 (*10563*)
	Ovine, adult	Chromaffin cells	Collagenase Type 2: 0.2% Deoxyribonuclease I: 100 u/ml	Locke's solution	Keating, D., Rychkov, G., Adams, M., Holgert, H., McMillen, I.C. and Roberts, M.: Opioid Receptor Stimulation Suppresses the Adrenal Medulla Hypoxic Response in Sheep by Actions on Ca(2+) and K(+) Channels., *J Physiol 555*, 489, 2004 (*10567*)
	Ovine, 3 year	Anterior pituitary	Trypsin: 2.5% Deoxyribonuclease I: 0.004%	DMEM	Canny B J, O'Farrell K A, Clarke I J, Tilbrook A J: The influence of sex and gonadectomy on the hypothalamo-pituitary-adrenal axis of the sheep, *J Endocrinol 162*, 215-25, 1999 (*10324*)
Rat	Rat, SD	Chromaffin cells	Collagenase Type 1: 0.26% Deoxyribonuclease I: 0.015% Hyaluronidase: 0.015%	HBSS	Gilabert, J: Necessary Conditions to Maintain Rat Adrenal Chromaffin Cells in Primary Culture, *Cell Biology of the Chromaffin Cell*, Borges, R. and Gandia, L., Instituto Teofilo Hernando, 2004 (*10564*)
	Rat	Chromaffin	Collagenase Type 1: 0.26% Deoxyribonuclease I: 0.015% Hyaluronidase: 0.015%	HBSS	Gilabert, J, Montalvo, G, and Artalejo A.: Rat Chromaffin cells primary cultures: Standardization and quality assessment for single-cell assays, *Nat Protoc* , 294, 2006 (*10349*)
	Rat, SD, male	Zona fasciculata/reticularis	Collagenase: 0.4%	Krebs-HEPES	Bruder Eric D, Ball Dennis L, Goodfriend Theodore L, Raff Hershel: An oxidized metabolite of linoleic acid stimulates corticosterone production by rat adrenal cells, *Am J Physiol Regul Integr Comp Physiol 284*, R1631-5, 2003 (*10134*)
	Rat, Wistar, newborn	Chromaffin cells	Collagenase Type 1: 0.025% Deoxyribonuclease I: 0.0015%	DMEM	Zhang L, Castell A, Avila E, Drucker-Col√≠n R, Escobar A: Immunocytochemical, ultrastructural and neurochemical evidences on synaptogenesis and dopamine release of rat chromaffin cells co-cultured with striatal neurons, *J Neuropathol Exp Neurol 59*, 170-4, 2000 (*10247*)
	Rat, SD, male	ZG ZFR	Collagenase Type 1: 0.2%	Kreb's	Sayed, S., Whitehouse, B., and Jones, P.: Phosphoserine/Threonine Phosphatases in the Rat Adrenal Cortex: A Role in the Control of Steroidogenesis, *J Endocrinol 154*, 449, 1997 (*1072*)
	Rat, Fischer, male, 10-16 weeks	Adrenocortical	Deoxyribonuclease I: 0.005%	BSS	Roskelley, C.D. and Auersperg, N.: Density Separation of Rat Adrenocortical Cells: Morphology, Steroidogenesis, and P-450scc Expression in Primary Culture, *In Vitro Cell Dev Biol 26*, 493, 1990 (*425*)
	Rat, male, 120-160 g, Rat, SD, male, 400-450 g	Leydig Adrenal	Collagenase Type 2: 0.03% (adrenal)	Krebs Ringer bicarbonate buffer	Ng, T. and Liu, W.: Toxic Effect of Heavy Metals on Cells Isolated from the Rat Adrenal and Testis, *In Vitro Cell Dev Biol 26*, 24, 1990 (*435*)
	Rat, SD, 2-4 day old (also bovine)	Heart Adrenal chromaffin Paraneurons	Trypsin: 0.06%	25mM HEPES buffered Locke's solution, CMF	Trifaro, J., Tang, R., and Novas, M.: Monolayer Co-Culture of Rat Heart Cells and Bovine Adrenal Chromaffin Paraneurons, *In Vitro Cell Dev Biol 26*, 335, 1990 (*438*)
	Rat, Long-Evans, female, 150-200 g	Glomerulosa	Collagenase: 0.2%	MEM-d-Val	Payet, N., Deziel, Y., and Lehoux, J.-G.: Vasopressin: A Potent Growth Factor in Adrenal Glomerulosa Cells in Culture, *J Steroid Biochem 20*, 449, 1984 (*621*)
	Rat, Fischer, male, 1-10 months	Adrenocortical	Deoxyribonuclease I: 0.005%	BSS	Leonard, R.K., Auersperg, N., and Parkes, C.O.: Ascorbic Acid Accumulation by Cultured Rat Adrenocortical Cells, *In Vitro 19*, 46, 1983 (*527*)
	Rat, SD, male, 400-450 g	Decapular Capsular Glomerulosa	Deoxyribonuclease I: 0.01%	Medium 199	Li, C.H., Ng, T.B., and Cheng, C.H.K.: Melanotropins: Aldosterone- and Corticosterone-Stimulating Activity in Isolated Rat Adrenal Cells, *Int J Pept Protein Res 19*, 361, 1982 (*543*)
	Rat	Chromaffin	Trypsin: 0.10%	Ham's F-12 w/HEPES	Englert, D.F.: An Optical Study of Isolated Rat Adrenal Chromaffin Cells, *Exp Cell Res 125*, 369, 1980 (*389*)
	Rat, Hanover-Wistar, 2nd postnatal week (also guinea-pig, cattle)	Chromaffin	Collagenase: 0.5%	HBSS	Unsicker, K., Rieffert, B., and Ziegler, W.: Effects of Cell Culture Conditions, Nerve Growth Factor, Dexamethasone, and Cyclic AMP on Adrenal Chromaffin Cells, *Adv Biochem Psychopharmacol 25*, 51, 1980 (*711*)
	Rat , SD, female, 200 g	Glomerulosa	Deoxyribonuclease I: 0.05%	Kreb's	Braley, L., Williams, G., and Bradwin, G.: The Effect of Unit Gravity Sedimentation on Adrenal Steroidogenesis by Isolated Rat Glomerulosa and Fasciculata Cells, *Endocrinology 106 (1)*, 50, 1980 (*769*)
	Rat	Foreskin	Collagenase: 0.5%	Dulbecco's MEM w/10% calf serum	Folkman, J., Haudenschild, C. C., and Zetter, B. R.: Long-term Culture of Capillary Endothlial Cells, *Proc Natl Acad Sci U S A 76*, 5217, 1979 (*653*)
	Rat, Wistar-Hanover, 7-12 day	Medullary	Trypsin: 0.125%	HBSS	Unsicker, K., Krisch, B., Otten, U., and Thoenen, H.: Nerve Growth Factor-Induced Fiber Outgrowth From Isolated Rat Adrenal Chromaffin Cells: Impairment by Glucocorticoids , *Proc Natl Acad Sci U S A 75 (7)*, 3498, 1978 (*988*)
	Rat, SD, male	Cortical	Trypsin: 0.25%	Kreb's Ringer bicarbonate buffer	Barofsky, A., Feinstein, M., and Halkerston, I.: Enzymatic and Mechanical Requirements for the Dissociation of Cortical Cells From Rat Adrenal Glands, *Exp Cell Res 79*, 263, 1973 (*1010*)
	Rat, Holtzman, male, 180-250 g	Adrenal	Collagenase Type 1: 0.5%	Kreb's Ringer bicarbonate buffer	Kloppenborg, P., Island, D., Liddle, G., Michelakis, A., and Nicholson, W.: A Method of Preparing Adrenal Cell Suspensions and Its Applicability to the *In Vitro* Study of Adrenal Metabolism, *Endocrinology 82*, 1053, 1968 (*383*)

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Bone

Species		Cell(s)	Enzyme(s)	Medium	Reference
Bovine	Bovine	Chondrocytes	Collagenase Type 2: 0.4%	DMEM	Buschmann, M., Gluzband, Y., Grodzinsky, A., and Hunziker, E.: Mechanical Compression Modulates Matrix Biosynthesis in Chondrocyte/Agarose Culture, *J Cell Sci 108*, 1497, 1995 (*1133*)
Chicken	Chick, day old	Osteoblasts	Trypsin: 0.03%	DMEM	Gay, C., Lloyd, Q., and Gilman, V.: Characteristics and Culture of Osteoblasts Derived From Avian Long Bone, *In Vitro Cell Dev Biol 30A*, 379, 1994 (*1036*)
Human	Human, 22-73 year	Osteoblasts	Trypsin: 0.5%	Basal Medium	Kneser U, Voogd A, Ohnolz J, Buettner O, Stangenberg L, Zhang YH, Stark GB, Schaefer DJ: Fibrin gel-immobilized primary osteoblasts in calcium phosphate bone cement: in vivo evaluation with regard to application as injectable biological bone substitute, *Cells Tissues Organs 179*, 158-69, 2005 (*10316*)
Mouse	Mouse	Endosteal cells	Collagenase Type 1: 0.3%	DMEM	Nakamura, Y., Arai, F., Iwasaki, H., Hosokawa, K., Kobayashi, I., Gomei, Y., Matsumoto, Y., Yoshihara, H. and Suda, T.: Isolation and Characterization of Endosteal Niche Cell Populations that Regulate Hematopoietic Stem Cells., *Blood 116*, 1422, 2010 (*10621*)
	Mouse, Swiss-Webster	Neonatal bone	Collagenase Type 2: 0.20%	Tris-buffered saline	Chen,T. and Feldman,D.: Regulation of 1,25-Dihydroxyvitamin D3 Receptors in Cultured Mouse Bone Cells, *J Biol Chem 256*, 5561, 1981 (*554*)
Rat	Rat, fetal, 21 days of gestation	Calvaria	Collagenase: 0.2%	MEM	Owen, T., Aronow, M., Shalhoub, V., Barone, L., Wilming, L., Tassinari, M., Kennedy, M., Pockwinse, S., Lian, J., and Stein, G.: Progressive Development of the Rat Oseoblast Phenotype *In Vitro*: Reciprocal Relationships in Expression of Genes Associated with Osteoblast Proliferation and Differentiation During Formation of the Bone Extracellular Matrix, *J Cell Physiol 143*, 420, 1990 (*1235*)
	Rat, newborn	Osteoblastlike cells	Collagenase Type 2: 0.3%	MEM	Ernst, M., and Froesch, E.: Osteoblastlike Cells in a Serum-Free Methylcellulose Medium Form Colonies: Effects of Insulin and Insulinlike Growth Factor I, *Calcif Tissue Int 40*, 27, 1987 (*1007*)

Brain

Species		Cell(s)	Enzyme(s)	Medium	Reference
Bovine	Bovine	Microvascular endothelial	Neutral Protease: 0.005%	Medium 199	Kanda, T., Yoshino, H., Ariga, T., Yamawaki, M., and Yu, R.: Glycosphingolipid Antigens in Cultured Microvasular Bovine Brain Endothelial Cells: Sulfoglucuronosyl Paragloboside as a Target of Monoclonal IgM in Demyelinative Neuropathy, *J Cell Biol 126 (1)*, 235, 1994 (*950*)
	Bovine	Brain endothelial cells	Collagenase Type 2: 0.35%	DMEM	Wolburg H, Neuhaus J, Kniesel U, Krauss B, Schmid EM, Ocalan M, Farrell C, Risau W: Modulation of tight junction structure in blood-brain barrier endothelial cells. Effects of tissue culture, second messengers and cocultured astrocytes, *J Cell Sci 107*, 1347, 1994 (*10048*)
	Bovine	Endothelial	Neutral Protease: 0.125%	MEM	Miller, D., Audus, K., and Borchardt, R.: Application of Cultured Endothelial Cells of the Brain Microvasculature in the Study of the Blood-Brain Barrier, *J Tiss Cul Meth 14*, 217, 1992 (*942*)
	Bovine, adult	Cerebral artery Endothelial	Collagenase: 0.2%	HBSS	Machi, T., Kassell, N.F., and Scheld, W.M.: Isolation and Characterization of Endothelial Cells From Bovine Cerebral Arteries, *In Vitro Cell Dev Biol 26*, 291, 1990 (*436*)
	Calf (also lamb)	Oligodendroglia Neural	Trypsin: 0.1%	(see reference)	Poduslo, S., Miller, K., and McKhann, G.: Metabolic Properties of Maintained Oligodendroglia Purified from Brain, *J Biol Chem 253*, 1592, 1978 (*552*)
Guinea-Pig	Guinea-pig, 200-400 g	Neurons	Trypsin: 0.06-0.08%	PIPES saline	Kay, A.R., and Wong, R.K.S.: Isolation of Neurons Suitable for Patch-Clamping from Adult Mammalian Central Nervous Systems, *J Neurosci Methods 16*, 227, 1986 (*607*)
Human	Human	Tumor	Collagenase Type 4: 0.1% Hyaluronidase: 0.07% Deoxyribonuclease I: 0.04%	(see reference)	Sauvageot, C., Weatherbee, J., Kesari, S., Winters, S., Barnes, J., Dellagatta, J., Ramakrishna, N., Stiles, C., Kung, A., Kieran, M. and Wen, P.: Efficacy of the HSP90 Inhibitor 17-AAG in Human Glioma Cell Lines and Tumorigenic Glioma Stem Cells., *Neuro Oncol Vol. 11*, 109, 2009 (*10592*)
	Human	Microglia	Trypsin: 0.25% Deoxyribonuclease I: .005%	DMEM/F12	Klegeris Andis, McGeer PatrickL: Chymotrypsin-like proteases contribute to human monocytic THP-1 cell as well as human microglial neurotoxicity, *Glia 51*, 56-64, 2005 (*10112*)
	Human	Microvessels	Collagenase Type 4: 0.1%	DMEM	Gerhart, D. Z., Broderius, M. A., and Drewes, L. R.: Cutlured Human and Canine Endothelial Cells from Brain Microvessels, *Brain Res Bull 21*, 785, 1988 (*344*)
Insect	Drosophilia	Neurons	Papain: 20 u/ml	Saline	Gu, H. and O'Dowd, D.: Whole Cell Recordings from Brain of Adult Drosophila., *J Vis Exp 6*, 248, 2007 (*10651*)
Monkey	Monkey, rhesus	Brain cells	Collagenase Type 2: 500 u/ml Deoxyribonuclease I: 28 u/ml	HBSS	Marcondes MC, Burudi EM, Huitron-Resendiz S, Sanchez-Alavez M, Watry D, Zandonatti M, Henriksen SJ, Fox HS: Highly activated CD8(+) T cells in the brain correlate with early central nervous system dysfunction in simian immunodeficiency virus infection, *J Immunol 167*, 5429-38, 2001 (*10125*)
Mouse	Mouse	Granule cell precursors, pre-neoplastic and tumor cells	Papain: 10 u/ml Deoxyribonuclease I: 250 u/ml	Neurobasal/B27	Oliver, T., Read, T., Kessler, J., Mehmeti, A., Wells, J., Huynh, T., Lin, S. and Wechsler-Reya, R.: Loss of Patched and Disruption of Granule Cell Development in a Pre-Neoplastic Stage of Medulloblastoma., *Development 132*, 2425, 2005 (*10555*)
	Mouse, SWR or CF1, 1-3 months	Papillae, taste receptor	Pronase E: 0.15%	Carbonate-Phosphate buffer (see reference)	Spielman, A., Mody, I., Brand, J., Whitney, G., MacDonald, J., and Salter, M.: A Method for Isolating and Patch-Clamping Single Mammalian Taste Receptor Cells, *Brain Res 503*, 326, 1989 (*350*)
	Mouse, 0-30 day	Neural	Trypsin NF 1:250: 50 0.25%	BSS	Shrier, B., Wilson, S., and Nirenberg, M.: Cultured Cell Systems and Methods for Neurobiology, *Vol. 32*, 765, 1974 (*637*)
Ovine	Lamb (also calf)	Oligodendroglia Neural	Trypsin: 0.1%	(see reference)	Poduslo, S., Miller, K., and McKhann, G.: Metabolic Properties of Maintained Oligodendroglia Purified from Brain, *J Biol Chem 253*, 1592, 1978 (*552*)
Porcine	Mini pigs, Yucatan (Susscrofa Yucatan), 4-6 months	Microvascular	Collagenase: 0.1%	HBSS	Robinson, D.H., Kang,Y., Deschner, S.H., and Nielsen, T.B.: Morphologic Plasticity and Periodicity : Porcine Cerebral Microvascular Cells in Culture, *In Vitro Cell Dev Biol 26*, 169, 1990 (*432*)

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Brain (Con't)

Brain (Con't)

Species		Cell(s)	Enzyme(s)	Medium	Reference
Rat	Rat, Fisher, 7-21 month	Hippocampal neurons	Papain: 0.2%	Hibernate A	Chen, N., Newcomb, J., Garbuzova-Davis, S., Davis Sanberg, C., Sanberg, P. and Willing, A.: Human Umbilical Cord Blood Cells Have Trophic Effects on Young and Aging Hippocampal Neurons in Vitro., *Aging Dis 1*, 173, 2010 (*10663*)
	Rat, SD, 7 day	Cerebellar granule neurons	PDS kit: per instructions	PBS	Tanaka, S., Shaikh, I., Chiocca, E. and Saeki, Y.: The Gs-Linked Receptor GPR3 Inhibits the Proliferation of Cerebellar Granule Cells During Postnatal Development., *PLoS ONE 4*, e5922, 2009 (*10487*)
	Rat, neonatal	Astrocytes	Papain: 20 u/ml	EBSS	Shigetomi, E. and Khakh, B.: Measuring Near Plasma Membrane and Global Intracellular Calcium Dynamics in Astrocytes., *J Vis Exp 26*, 1142, 2009 (*10656*)
	Rat, neonatal	Hippocampal neurons	Papain: 10 u/ml	EBSS	Richler Esther, Chaumont Severine, Shigetomi Eiji, Sagasti Alvaro, Khakh Baljit S: Tracking transmitter-gated P2X cation channel activation in vitro and in vivo, *Nat Methods 5*, 87-93, 2008 (*10319*)
	Rat, E18	Hippocampal neurons	Papain: 0.2%	Hibernate	Jekabsons MB, Nicholls DG: Bioenergetic analysis of cerebellar granule neurons undergoing apoptosis by potassium/serum deprivation, *Cell Death Differ 13*, 1595-610, 2006 (*10129*)
	Rat, Wistar, 1-3 day	Hippocampal neurons	Trypsin: 0.05%	DMEM	Velasco Myrian, Garcia Esperanza, Onetti Carlos G: Glucose deprivation activates diversity of potassium channels in cultured rat hippocampal neurons, *Cell Mol Neurobiol 26*, 307-19, 2006 (*10321*)
	Rat, E19	Hippocampal neuron	Papain: 10 u/ml	MEM	Khakh Baljit S, Fisher James A, Nashmi Raad, Bowser David N, Lester Henry A: An angstrom scale interaction between plasma membrane ATP-gated P2X2 and alpha4beta2 nicotinic channels measured with fluorescence resonance energy transfer and total internal reflection fluorescence microscopy, *J Neurosci 25*, 6911-20, 2005 (*10307*)
	Rat, Wistar, 14 day	Visual cortical	PDS kit:	EBSS	Mizoguchi Y, Kanematsu T, Hirata M, Nabekura J: A rapid increase in the total number of cell surface functional GABAA receptors induced by brain-derived neurotrophic factor in rat visual cortex, *J Biol Chem 278*, 44097, 2003 (*10022*)
	Rat, Wistar, male	Cerebral endothelial	Collagenase Type 3: 0.2%	MEM	Floris S, Van den Born J, van der Pol SM, Dijkstra CD, De Vries HE: Heparan sulfate proteoglycans modulate monocyte migration across cerebral endothelium, *J Neuropathol Exp Neurol 62*, 780, 2003 (*10041*)
Shellfish	*Helisoma trivolvis*	Neurons Buccal ganglia	Trypsin: 0.2%	Saline, sterile	Hadley, R.D., Bodnar, D.A., and Kater, S.B.: Formation of Electrical Synapses Between Isolated, Cultured Helisoma Neurons Requires Mutual Neurite Elongation, *J Neurosci 5*, 3145, 1985 (*615*)

Cartilage

Cartilage

Species		Cell(s)	Enzyme(s)	Medium	Reference
Bovine	Bovine, 18-36 month	Chondrocytes	Collagenase Type 1: 0.1%	HBSS	White, R. and Gibson, J.: The Effect of Oxygen Tension on Calcium Homeostasis in Bovine Articular Chondrocytes., *J Orthop Surg Res Vol. 5*, 27, 2010 (*10610*)
	Bovine, 6-8 week	Chondrocytes	Collagenase Type 2: 0.2%	DMEM	Hwang, Y., Sangaj, N. and Varghese, S.: Interconnected Macroporous Poly(ethylene glycol) Cryogels as a Cell Scaffold for Cartilage Tissue Engineering., *Tissue Eng Part A Vol. 16*,, , 3033-41, 2010 (*10631*)
	Steers, 1-2 years	Chondrocytes	Trypsin: 0.20%	HBSS	Mackintosh, D., and Mason, R.: Pharmacological Actions of 17 Beta-oestradiol on Articular Cartilage Chondrocytes and Chondrosarcoma Chondrocytes in the Absence of Oestrogen Receptors, *Biochim Biophys Acta 964*, 295, 1988 (*334*)
	Calf, 1-14 days	Chondrocytes	Collagenase Type 2: 0.20%	PBS	Klagsbrun, M.: Large Scale Preparation of Chondrocytes, *Vol. 58*,, , 560, 1979 (*1263*)
Chicken	Chick embryos, HH stage	Mesenchymal	Trypsin: 0.1%	DMEM (see reference)	Wong, M., and Tuan, R.: Nuserum, A Synthetic Serum Replacement, Supports Chondrogenesis of Embryonic Chick Limb Bud Mesenchymal Cells in Micromass Culture, *In Vitro Cell Dev Biol 29A*, 917, 1993 (*965*)
	Chicken, broiler strain, 8-10 weeks	Matrix vesicles Epiphyseal growth plate	Trypsin: 0.1%	Tris-buffered saline	Genge, B., Wu, L. and Wuthier, R.: Differential Fractionation of Matrix Vesicle Proteins: Further Characterization of the Acidic Phospholipid-dependent Ca^{2+}-Binding Proteins, *J Biol Chem 265*, 4703, 1990 (*569*)
	Chicken, broiler strain, 8-10 week	Matrix vesicles	Trypsin: 0.1%	(see reference)	Genge, B.R., Wu, L.N.Y., and Wuthier, R.E.: Identification of Phospholipid-dependent Calcium-binding Proteins as Constituents of Matrix Vesicles, *J Biol Chem 264*, 10917, 1989 (*564*)
Human	Human	Synoviocytes	Collagenase Type 1: 0.4%	DMEM	Kim Wan-Uk, Kwok Seung-Ki, Hong Kyung-Hee, Yoo Seung-Ah, Kong Jin-Sun, Choe Jongseon, Cho Chul-Soo: Soluble Fas ligand inhibits angiogenesis in rheumatoid arthritis, *Arthritis Res Ther 9*, R42, 2007 (*10173*)
	Human, 13-62 years	Articular chondrocytes	Trypsin: 0.2%	BSS	Srivastava, V.M.L., Malemud, C.J., Hough, A.J., Bland, J.H., and Sokoloff, L.: Preliminary Experience with Cell Culture of Human Articular Chondrocytes, *Arthritis Rheum 17*, 165, 1974 (*726*)
	Human, 26-84 years	Chondrocytes	Collagenase:	GBSS	Manning, W.K., and Bonner, W.M.: Isolation and Culture of Chondrocytes From Human Adult Articular Cartilage, *Arthritis Rheum 10*, 235, 1967 (*727*)
Mouse	Mouse, 1 day	Chondrocytes	Collagenase: 0.2%	DMEM	Terpstra, L, Prud'homme, J, Arabian, A, Takeda, S, Karsenty, G, Dedhar, S, and St-Arnaud, R.: Reduced Chondrocyte Proliferation and Chondrodysplasia in Mice Lacking the Integrin-linked Kinase in Chondrocytes, *J Cell Biol 162*, 139, 2003 (*9756*)
Ovine	Sheep, 2 month	Chondrocytes	Collagenase Type 2: 0.3%	Ham's F-12	Kojima Koji, Bonassar Lawrence J, Roy Amit K, Mizuno Hirokazu, Cortiella Joaquin, Vacanti Charles A: A composite tissue-engineered trachea using sheep nasal chondrocyte and epithelial cells, *FASEB J 17*, 823-8, 2003 (*10216*)
Porcine	Porcine, 1 year	Chondrocytes	Collagenase Type 1: 0.2%	DEMEM	Chowdhury, T., Schulz, R., Rai, S., Thuemmler, C., Wuestneck, N., Bader, A and Homandberg, G: Biomechanical Modulation of Collagen Fragment-Induced Anabolic and Catabolic Activities in Chondrocyte/Agarose Constructs., *Arthritis Res Ther 12*, R82, 2010 (*10611*)

[CF=Calcium Free • CMF=Calcium Magnesium Free • BSS=Balanced Salt Solution]

Cartilage (Con't)

Species		Cell(s)	Enzyme(s)	Medium	Reference
Porcine	Porcine, 2-4 month	Chondrons	Neutral Protease: 0.3% Collagenase: 0.2%	PBS	Graff RD, Lazarowski ER, Banes AJ, Lee GM: ATP release by mechanically loaded porcine chondrons in pellet culture, *Arthritis Rheum 43*, 1571-9, 2000 (*10253*)
Rabbit	Rabbit, New Zealand, white, 4-6 wk & 22-25 wk	Chondrocytes	Protease XIV: 5 mg/g of tissue	Ham's F-12	Plaas, A., Sandy, J., and Kimura, J.: Biosynthesis of Cartilage Proteoglycan & Link Protein Articular Chondrocytes, *J Biol Chem 263*, 7560, 1988 (*562*)
	Rabbit, white, male, 8 weeks	Chondrocytes	Trypsin: 0.2%	Gey's BSS	Benya, P.D., Padilla, S.R., and Nimni, M.E.: The Progeny of Rabbit Articular Chondrocytes Synthesize Collagen Types I and III and Type I Trimer, but Not Type II, *Biochem 16*, 865, 1977 (*312*)
	Rabbit, New Zealand white or Dutch, 1 week (also human, newborn)	Chondrocytes	Trypsin: 0.1%	Saline G, CMF	Schindler, F.H., Ose, M.A., and Solursh, M.: The Synthesis of Cartilage Collagen by Rabbit and Human Chondrocytes in Primary Cell Culture, *In Vitro 12*, 44, 1976 (*495*)
	Rabbit, New Zealand white, immature, 2.25 - 3.3 Kg	Articular chondrocytes Hyaline	Trypsin: 0.2%	Gey's BSS	Green, J.R., and William, T.: Articular Cartilage Repair. Behavior of Rabbit Chondrocytes During Tissue Culture and Subsequent Allografting, *Clin Orthop Relat Res* , 237, 1976 (*710*)
	Rabbit, New Zealand white, male, 250-350 g	Epiphyseal Articular cartilage	Trypsin: 0.25%	Eagle's basal medium	Bentley, G., and Greer , R.: Homotransplantation of Isolated Epiphyseal and Articular Cartilage Chondrocytes into Joint Surfaces of Rabbits, *Nature 230*, 385, 1971 (*641*)
Rat	Rat, SD, young, 100-120 g	Chondrocytes	Trypsin: 0.2%	Ham's F-12 medium	Shimomura, Y., Yoneda, T., and Suzuki, F.: Osteogenesis by Chondrocytes from Growth Cartilage of Rat Rib, *Calcif Tissue Res 19*, 179, 1975 (*351*)

Colon

Species		Cell(s)	Enzyme(s)	Medium	Reference
Human	Human	Cancer stem cells Enzyme(s)	Collagenase: Type 3: 200 u/ml Deoxyribonuclease 1: 100 u/ml	RPMI-1640	Dalerba P., Dylla S.J., Park I., Liu R., Wang X., Cho R. W., Hoey T., Gurney A., Huang E.H., Simeone D.M., Shelton A. A., Parmiani G., Castelli C., Clarke M.F: Phenotypic characterization of human colorectal cancer stem cells, *Proc Natl Acad Sci U S A 104*, 10158-63, 2007
Mouse	Mouse	Dendridic Enzyme(s)	Collagenase: 300 u/ml Deoxyribonuclease 1: .002% u/ml	RPMI-1640	Abe K., Nguyen K., Fine S.D., Mo Ji-Hun, Shen C., Shenouda S., Corr M., Jung S., Lee J., Eckmann L., Raz E.: Conventional dendritic cells regulate the outcome of colonic inflammation independently of T cells., *Proc Natl Acad Sci U S A 104*, 17022-7, 2007

Endothelial

Species		Cell(s)	Enzyme(s)	Medium	Reference
Bovine	Bovine, adult	Cerebral artery Endothelial	Collagenase: 0.2%	HBSS	Machi, T., Kassell, N.F., and Scheld, W.M.: Isolation and Characterization of Endothelial Cells From Bovine Cerebral Arteries, *In Vitro Cell Dev Biol 26*, 291, 1990 (*436*)
	Bovine	Endothelial Aortic	Trypsin: 0.05%	Krebs Ringer solution	DeNucci, G., Gryglewski, R.J., Warner, T.D., and Vane, J.R.: Receptor-Mediated Release of Endothelium-Derived Relaxing Factor and Prostacyclin From Bovine Aortic Endothelial Cells Is Coupled, *Proc Natl Acad Sci U S A 85*, 2334, 1988 (*659*)
	Bovine	Endothelial, pulmonary artery	Collagenase: 0.1%	CMF-Dulbecco's PBS	Martin, T.: Formation of Diacylglycerol by a Phospholipase D-phosphatidate Phosphatase Pathway Specific for Phosphatidylcholine in Endothelial Cells, *Biochim Biophys Acta 962*, 282, 1988 (*333*)
	Bovine	Endothelial Aorta	Collagenase Type 2: 0.1%	PBS	Carson, M.P. and Haudenschild, C.C.: Microvascular Endothelium and Pericytes: High Yield, Low Passage Cultures, *In Vitro Cell Dev Biol 22*, 344, 1986 (*417*)
	Bovine	Endothelial Brain arteries	Collagenase Type 2: 0.2%	Dulbecco's PBS	Goetz, I.., Warren, J., Estrada, C., Roberts, E., and Krause, D.: Long-Term Cultivation of Arterial and Capillary Endothelium From Adult Bovine Brain, *In Vitro Cell Dev Biol 21*, 172, 1985 (*413*)
	Bovine	Endothelial Corneal	Trypsin: 0.2%	PBS: DMEM	Scott, D., Murray, J., and Barnes, M.: Investigation of the Attachment of Bovine Corneal Endothelial Cells, *Exp Cell Res 144*, 472, 1983 (*393*)
	Bovine, 2-3 weeks	Endothelial Pulmonary artery	Collagenase Type 1: 0.2%	RPMI 1640 w/1% Fetal Bovine Serum	Lee, S., Douglas, W., Deneke, S., and Fanburg, B.: Ultrastructural Changes in Bovine Pulmonary Artery Endothelial Cells Exposed to 80% O_2 *In Vitro*, *In Vitro 19*, 714, 1983 (*531*)
	Bovine	Endothelial, corneal	Trypsin: 0.05%	0.01M Phosphate buffer with 0.02% EDTA, 0.9% NaCl (see reference)	Robinson, J. and Gospodarowicz, D.: Glycosaminoglycans Synthesized by Cultured Bovine Corneal Endothelial Cells, *J Cell Physiol 117*, 368, 1983 (*594*)
	Bovine	Endothelial Subclavian vein	Collagenase Type 1: 0.10%	PBS	Olander, J., Marasa, J., Kimes, R., Johnston, G., and Feder, J.: An assay measuring the stimulation of several types of bovine endothelial cells by growth factor(s) derived from cultured human tumor cells, *In Vitro 18*, 99, 1982 (*525*)
	Bovine	Aortic Pulmonary artery	Collagenase Type 2: 0.10%	PBS	Makarski, J. S.: Stimulation of cyclic AMP production by vasoactive agents in cultured bovine aortic and pulmonary artery endothelial cells, *In Vitro 17*, 450, 1981 (*513*)
	Calf, fetal, 4-9 months	Endothelial	Collagenase Type 1: 0.25%	PBS	Rosen, E., Mueller, S., Noveral, J., and Levine, E.: Proliferative Characteristics of Clonal Endothelial Cell Strains, *J Cell Physiol 107*, 123, 1981 (*880*)
	Bovine, adult	Aorta	Collagenase Type 1: 125 u/ml	Dulbecco's PBS with calcium and magnesium	Cotta-Pereira, G., Sage, H., Bornstein, P., Ross, R., and Schwartz, S.: Studies of Morphologically Atypical ("Sprouting") Cultures of Bovine Aortic Endothelial Cells. Growth Characteristics and Connective Tissue Protein Synthesis, *J Cell Physiol 102*, 183, 1980 (*592*)
	Bovine, young	Pulmonary artery	Collagenase: 0.1%	Medium 199	Ryan, U., Mortara, M., and Whitaker, C.: Methods for Microcarrier Culture of Bovine Pulmonary Artery Endothelial Cells Avoiding the Use of Enzymes, *Tissue Cell 12*, 619, 1980 (*670*)
	Bovine	Foreskin	Collagenase: 0.5%	Dulbecco's MEM w/10% calf serum	Folkman, J., Haudenschild, C. C., and Zetter, B. R.: Long-term Culture of Capillary Endothlial Cells, *Proc Natl Acad Sci U S A 76*, 5217, 1979 (*653*)
	Bovine	Saphenous Vein Aorta	Collagenase: 0.01%	PBS	Eskin, S., Sybers, H., Trevino, L., Lie, J., and Chimoskey, J.: Comparison of Tissue-Cultured Bovine Endothelial Cells from Aorta and Saphenous Vein, *In Vitro 14*, 903, 1978 (*500*)

[CF=Calcium Free • CMF=Calcium Magnesium Free • BSS=Balanced Salt Solution]

Endothelial (Con't)

Species		Cell(s)	Enzyme(s)	Medium	Reference
Bovine	Bovine	Pulmonary artery	Collagenase Type 2: 0.25%	Puck's solution	Ryan, U.S., Clements, E., Habliston, D., and Ryan, J.W.: Isolation And Culture of Pulmonary Artery Endothelial Cells, *Tissue Cell 10*, 535, 1978 (*669*)
	Calf	Endothelial	Collagenase Type 2: 0.1%	DMEM	Howard, B., Macarak, E., Gunson, D., and Kefalides, N.: Characterization of the Collagen Synthesized by Endothelial Cells in Culture, *Proc Natl Acad Sci U S A 73 (7)*, 2361, 1976 (*954*)
Canine	Dog (also human)	Microvessels	Collagenase Type 4: 0.1%	DMEM	Gerhart, D. Z., Broderius, M. A., and Drewes, L. R.: Cutlured Human and Canine Endothelial Cells from Brain Microvessels, *Brain Res Bull 21*, 785, 1988 (*344*)
	Dog, mongrel, adult	Endothelium Jugular vein	Trypsin: 0.1%	Earle's PBS, CMF	Ford, J., Burkel, W., and Kahn, R.: Isolation of Adult Canine Venous Endothelium for Tissue Culture, *In Vitro 17*, 44, 1981 (*512*)
Guinea-Pig	Guinea-Pig, female 300-350g	Coronary endothelial	Collagenase Type 2: 0.1%	(see reference)	Buxton I L, Kaiser R A, Oxhorn B C, Cheek D J: Evidence supporting the Nucleotide Axis Hypothesis: ATP release and metabolism by coronary endothelium, *Am J Physiol Heart Circ Physiol 281*, H1657-66, 2001 (*10171*)
Human	Human	Endothelial and vascular smooth muscle	Collagenase Type 1: 0.2%	HBSS	Moss, S., Bates, M., Parrino, P. and Woods, TC.: Isolation of Endothelial Cells and Vascular Smooth Muscle Cells from Internal Mammary Artery Tissue., *Ochsner J 7*, 133, 2007 (*10636*)
	Human	Endothelial	Collagenase Type 1: 0.2%	DMEM	Patel, V., Logan, A., Watkinson, J., Uz-Zaman, S., Sheppard, M., Ramsden, J. and Eggo, M.: Isolation and Characterization of Human Thyroid Endothelial Cells., *Am J Physiol Endocrinol Metab Vol. 284*, E168, 2003 (*10586*)
	Human, 18-68 yr	Corneal endothelial	Collagenase: 0.2% Neutral Protease: 1.0%	DMEM/F12	Li Wei, Sabater Alfonso L, Chen Ying-Ting, Hayashida Yasutaka, Chen Szu-Yu, He Hua, Tseng Scheffer C G: A novel method of isolation, preservation, and expansion of human corneal endothelial cells, *Inv Ophthal Visual Sci 48*, 614-20, 2007 (*10306*)
	Human	HUVEC	Collagenase Type 4: 0.1%	RPMI 1640	Silva AP, Kaufmann JE, Vivancos C, Fakan S, Cavadas C, Shaw P, Brunner HR, Vischer U, and Grouzmann E.: Neuropeptide Y expression, localization and cellular transducing effects in HUVEC, *Biol Cell 97(6)*, 457, 2005 (*9816*)
	Human	Colonic endothelial cells	Collagenase Type 2: 0.25%	HBSS/5%FBS	Wang D., Lehman R., Donner D., Matli M., Warren R., and Welton M.: Expression and Endocytosis of VEGF and Its Receptors in Human Colonic Vascular Endothelial Cells, *Am J Physiol/Gastro 282*, G1088, 2002 (*9817*)
	Human	Pulmonary vascular endothelial cells	Neutral Protease: 1.18 u/ml Elastase: 10 u/ml	M199	Muller AM, Hermanns MI, Skrzynski C, Nesslinger M, Muller KM, and Kirkpatrick CJ.: Expression of the endothelial markers PECAM-1, vWf, and CD34 in vivo and in vitro, *Exp Mol Pathol 72*, 221, 2002 (*9823*)
	Human	HUVEC	Collagenase Type 2: 0.1%	PBS	Takano Manabu, Meneshian Avedis, Sheikh Emran, Yamakawa Yasuhiko, Wilkins Kirsten Bass, Hopkins Elise A, Bulkley Gregory B: Rapid upregulation of endothelial P-selectin expression via reactive oxygen species generation, *Am J Physiol Heart Circ Physiol 283*, H2054-61, 2002 (*10311*)
	Human	Endothelial	Trypsin: 2%	PBS	Goolcharran, C., Cleland, J., Keck, R., Jones, A., and Borchardt, R.: Comparsion of the Rates of Deamidation, Diketopiperazine Formation and Oxidation in Recombinant Human Vascular Endothelial Growth Factor and Model Peptides, *AAPS PharmSci 2 (1)*, 5, 2000 (*742*)
	Human	HUVEC, porcine pulmonary arterial endothelial cells	Collagenase Type 2: 0.2%	DMEM	Kwak HJ, Lee SJ, Lee YH, Ryu CH, Koh KN, Choi HY, and Koh GY.: Angiopoietin-1 inhibits irradiation- and mannitol-induced apoptosis in endothelial cells, *Circulation 101(19)*, 2317, 2000 (*9818*)
	Human	Vascular endothelial cells	Collagenase: 0.1%	DMEM	Schonbeck U, Sukhova GK, Graber P, Coulter S, Libby P: Augmented expression of cyclooxygenase-2 in human atherosclerotic lesions, *Am J Pathol 155*, 1281-91, 1999 (*10343*)
	Human	Hepatic endothelial cells	Collagenase: 0.2%	DMEM	Sanyal AJ, and Mirshahi F.: A simplified method for the isolation and culture of endothelial cells from pseudointima of transjugular intrahepatic portasystemic shunts, *Lab Invest 78(11)*, 1469, 1998 (*9819*)
	Human	Umbilical vein HUVE	Collagenase: 0.1%	Cord buffer (see reference)	Grant, D.S., Lelkes, P.I., Fukuda, K., Kleinman, H.K.: Intracellular Mechanisms Involved in Basement Membrane Induced Blood Vessel Differentiation *In Vitro*, *In Vitro Cell Dev Biol 27*, 327, 1991 (*462*)
	Human	Microvessels	Collagenase Type 4: 0.1%	DMEM	Gerhart, D. Z., Broderius, M. A., and Drewes, L. R.: Cutlured Human and Canine Endothelial Cells from Brain Microvessels, *Brain Res Bull 21*, 785, 1988 (*344*)
	Human	Endothelial/HUVE Foreskin & umbilical cord	Trypsin: 0.3%	HBSS/PBS, Medium 199 (see reference)	Kubota, Y., Kleinman, H., Martin, G., and Lawley, T.: Role of Laminin and Basement Membrane in Morphological Differentiation of Human Endothelial Cells into Capillary-like Structures, *J Cell Biol 107*, 1589, 1988 (*580*)
	Human	Umbilical cord Smooth muscle	Collagenase: 0.1%	HEPES	Hoshi, H., Kan, M., Chen, J., and McKeehan, W.: Comparative Endocrinology-Paracrinology-Autocrinology of Human Adult Large Vessel Endothelial and Smooth Muscle Cells, *In Vitro Cell Dev Biol 24 (4)*, 309, 1988 (*937*)
	Human	Endothelial Saphenous vein	Collagenase Type 2: 0.1%	PBS, CMF	Sharefkin, J.B., Fairchild, K.D., Albus, R.A., Cruess, D.F., and Rich, N.M.: The Cytoxic Effect of Surgical Glove Powder Particles on Adult Human Vascular Endothelial Cell Cultures: Implications for Clinical Uses of Tissue Culture Techniques, *J Surg Res 41*, 463, 1986 (*725*)
	Human	Endothelial Dermal	Trypsin: 0.3%	PBS	Marks, R.M., Czerniecki, M., and Penny, R.: Human Dermal Microvascular Endothelial Cells: An Improved Method for Tissue Culture and Description of Some Singular Properties in Culture, *In Vitro Cell Dev Biol 21*, 627, 1985 (*415*)
	Human	Fibroblasts Foreskin	Hyaluronidase: 0.10%	DMEM	Gordon, P., Sussman, I., and Hatcher, V.: Long-Term Culture of Human Endothelial Cells, *In Vitro 19*, 661, 1983 (*530*)
	Human	Iliac arteries	Collagenase: 0.25%	PBS w/Ca++, Mg++, & BSA (see reference)	Glassberg, M., Bern, M., Coughlin, S., Haudenschild, C., Hoyer, L., and Antoniades, H.: Cultured Endothelial Cells Derived from the Human Iliac Arteries, *In Vitro 18*, 859, 1982 (*524*)

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Endothelial (Con't)

Endothelial (Con't)

Species		Cell(s)	Enzyme(s)	Medium	Reference
Human	Human (adult and child)	Foreskin	Collagenase: 0.5%	Dulbecco's MEM w/10% calf serum	Folkman, J., Haudenschild, C. C., and Zetter, B. R.: Long-term Culture of Capillary Endothlial Cells, *Proc Natl Acad Sci U S A 76*, 5217, 1979 (*653*)
	Human	Umbilical vein	Trypsin: 100 µg/ml	Tris-HCl,0.2 M	Jaffe, E.A., Minick, C.R., Adelman, B., Becker, C.G., and Nachman, R.: Synthesis of Basement Membrane Collagen By Cultured Human Endothelial Cells, *J Exp Med 144*, 209, 1976 (*602*)
	Human	Umbilical vein	Collagenase Type 1: 125 u/ml	Dulbecco's PBS	Gimbrone Jr., M.A.: Culture of Vascular Endothelium, *Prog Hemost Thromb 3*, 1, 1976 (*709*)
	Human	Umbilical cord	Collagenase: 0.1%	Dulbecco's PBS	Gimbrone, M.A., Cotran, R.S., and Folkman, J.: Human Vascular Endothelial Cells in Culture: Growth and DNA Synthesis, *J Cell Biol 60*, 673, 1974 (*589*)
	Human	Umbilical cord	Collagenase: 0.2%	Cord buffer (see reference)	Jaffe, E., Nachman, R., Becker, C., and Minick, C.: Culture of Human Endothelial Cells Derived from Umbilical Veins. Identification by Morphologic and Immunologic Criteria, *J Clin Invest 52*, 2745, 1973 (*598*)
	Human	Umbilical cord	Trypsin NF 1:250: 0.125%	Saline, normal	Lewis, L.J., Haok, J.C., Maca, R.D., and Fry, G.L.: Replication of Human Endothelial Cells in Culture, *Science 181*, 452, 1973 (*666*)
	Human	Umbilical cord	Trypsin NF 1:250: 0.25%	CMF solution	Fryer, D.G., Birnbaum, G., and Luttrell, C.N.: Human Endothelium in Cell Culture, *J Atheroscler Res 6*, 151, 1966 (*547*)
Mouse	Mouse, 7-10 week	Liver endothelial	Collagenase: 0.03%	DMEM	Follenzi, A., Benten, D., Novikoff, P., Faulkner, L., Raut, S. and Gupta, S.: Transplanted Endothelial Cells Repopulate the Liver Endothelium and Correct the Phenotype of Hemophilia A Mice., *J Clin Invest 118*, 935, 2008 (*10632*)
Porcine	Porcine	Endothelial Aortic	Collagenase Type 2: 0.1%	Dulbecco-Vogt MEM w/o serum	Dickinson, E. and Slakey, L.: Plasma-derived Serum as a Selective Agent to Obtain Endothelial Cultures from Swine Aorta, *In Vitro 18*, 63, 1982 (*523*)
	Porcine, 30-40 kg	Endothelia Aortic	Collagenase Type 4: 0.025%	Medium 199	Merrilees, M.J., and Scott, L.: Interaction of Aortic Endothelial and Smooth Muscle Cells in Culture, *Atherosclerosis 39*, 147, 1981 (*307*)
	Porcine, 20-30 week	Endothelial Aortas Veins	Collagenase: 0.1%	Medium 199 w/BSS & HEPES or $NaHCO_3$	Slater, D.N., and Sloan, J.M.: The Porcine Endothelial Cell in Tissue Culture, *Atherosclerosis 21*, 259, 1975 (*305*)
	Porcine, 60-100 days	Aorta	Trypsin: 0.1%	Phosphate buffer (see reference)	Coulson, W.F.: The Effect Of Proteolytic Enzymes on the Tensile Strength of Whole Aorta and Isolated Aortic Elastin, *Biochim Biophys Acta 237*, 378, 1971 (*319*)
Rabbit	Rabbit, 2-3Kg	Endothelial, aortic	Elastase: 0.2%	Hanks solution	Haley, N., Shio, H., Fowler, S.: Characterization of lipid-laden aortic cells from cholesterol-fed rabbits. I. Resolution of aortic cell populations by metrizamide density gradient centrifugation, *Lab Invest 37*, 287, 1977 (*624*)
Rat	Rat, SD, male, 250-300 g	Smooth muscle, aorta	Soybean Trypsin Inhibitor: 0.25%	HBSS with 0.2 mM Ca++	Schwertschlag, U.S., and Whorton, A.R.: Platelet-Activating Factor-Induced Homologous and Heterologous Desensitization In Cultured Vascular Smooth Muscle Cells, *J Biol Chem 263*, 13791, 1988 (*560*)
	Rat, SD, male, 350 - 450 g	Lipocytes Kupffer Sinusoidal endothelial	Collagenase: 0.015%	DMEM/Ham's F-12	Friedman, S. and Roll, F.: Isolation and Culture of Hepatic Lipocytes,Kupffer Cells, and Sinusoidal Endothelial Cells by Density Gradient Centrifugation with Stractan, *Anal Biochem 161*, 207, 1987 (*301*)
	Rat, Wistar, male, 3 mo	Endothelial Kupffer Parenchymal	Pronase: 0.25%	HBSS	Nagelkenke, J., Barto, K., and VanBerkel, T.: In vivo and in vitro uptake and degradation of acetylated low density lipoprotein by rat liver endothelial, Kupffer, and parenchymal cells, *J Biol Chem 258*, 12221, 1983 (*557*)
	Rat, Wistar-Kyoto, male, 100 - 200 g	Endothelial Cerebral	Collagenase Type 2: 0.05%	HBSS	Diglio, C.A., Grammas, P., Filiberto Giacomelli, M.S., and Wiener, J.: Primary Culture of Rat Cerebral Microvascular Endothelial Cells, *Lab Invest 46*, 554, 1982 (*626*)
	Rat, 300 G, and pig, 30-40 Kg	Endothelial Thoracic aorta	Trypsin: 0.05%	Medium 199 and 0.01M EDTA	Merrilees, M.J., and Scott, L.: Interaction of aortic endothelial and smooth muscle cells in culture. Effect on glycosaminoglycan levels, *Atherosclerosis 39*, 147, 1981 (*306*)
	Rat	Foreskin	Collagenase: 0.5%	Dulbecco's MEM w/10% calf serum	Folkman, J., Haudenschild, C. C., and Zetter, B. R.: Long-term Culture of Capillary Endothlial Cells, *Proc Natl Acad Sci U S A 76*, 5217, 1979 (*653*)
	Rat, Wistar, adult, 170 g	Endothelial	Trypsin: 0.5%	BSS	Phillips, P., Kumar, P., Kumar, S., and Waghe, M.: Isolation And Characterization of Endothelial Cells From Rat And Cow Brain White Matter, *J Anat 129*, 261, 1979 (*708*)

Epithelial

Epithelial

Species		Cell(s)	Enzyme(s)	Medium	Reference
Bovine	Bovine, fetal	Epithelial Tracheal	Neutral Protease: 2%	Dissociation medium, CMF	Schumann, B.L., Cody, T.E., Miller, M.L., Leikauf, G.D.: Isolation, Characterization, and Long-Term Culture of Fetal Bovine Tracheal Epithelial Cells, *In Vitro Cell Dev Biol 24*, 211, 1988 (*422*)
Canine	Dog	Tracheal	Pronase: 0.1%	DMEM	Virmani, A., Naziruddin, B., Desai, V., Lowry, J., Graves, D., and Sachdev, G.: Evidence for Secretion of High Molecular Weight Mucins by Canine Tracheal Epithelial Cells in Primary Culture: Effects of Select Secretagogues on Mucin Secretion, *In Vitro Cell Dev Biol 28A*, 120, 1992 (*1194*)
Chicken	Chick, 5 day old	Intestinal mesenchymal and epithelial	Collagenase: 0.03%	DMEM	Simon-Assmann, P and Kedinger, M: Embryonic Gut-Dissagregated Culture,*Cell & Tissue Culture: Laboratory Procedures Vol. 1*,Doyle, A., Griffiths, J., and Newell, D., John Wiley and Sons, Ltd., 12A:3.1, 1995 (*1271*)
Fish	Shark (*Squalus acanthias*)	Rectal gland	Collagenase: 0.2%	Ringer's solution	Karnaky, Jr., K.J., Valentich, J.D., Currie, M.G., Oehlenschlager, W.F., and Kennedy, M.P.: Atriopeptin Stimulates Chloride Secretion in Cultured Shark Rectal Gland Cells, *Am J Physiol 260*, 1125, 1991 (*287*)
	Shark (*Squalus acanthias*)	Rectal gland	Collagenase: 0.2%	Ringer's solution	Valentich, J: , *J Tiss Cul Meth 13*, 149, 1991 (*1265*)

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Species		Cell(s)	Enzyme(s)	Medium	Reference
Guinea-Pig	Guinea-pig, Hartley albino, 500-600 g	Endometrial	Collagenase Type 1: 0.1%	HBSS	Chaminadas, G., Alkhalaf, M., Remy-Martin, J.P., Propper, A.Y., and Adessi, G.L.: Specific Effect of Oestrone Sulphate on Protein Synthesis and Secretion by Cultured Epithelial Cells from Guinea-pig Endometrium, *J Endocrinol 123*, 233, 1989 (*600*)
Hamster	Hamster, Syrian gold, male, 100-120 g	Tracheal	Pronase: 0.1%	MEM with Hepes, CMF	Niles,R., Kim, K.C., Hyman, B., Christensen, T., Wasano, K., Brody, J.: Characterization Of Extended Primary And Secondary Cultures Of Hamster Tracheal Epithelial Cells, *In Vitro Cell Dev Biol 24*, 457, 1988 (*423*)
	Hamster, Syrian golden (strain CR:RGH)	Tracheal	Trypsin: 0.1%	Ham's F-12	McDowell, E., et al.: Differentiation of Tracheal Mucociliary Epithelium in Primary Cell Culture Recapitulates Normal Fetal Development and Regeneration Following Injury in Hamsters, *Am J Pathol 129*, 511, 1987 (*283*)
	Hamster (strain 1516 EHS and Lakeview), 8-12 weeks	Tracheal	Trypsin: 0.05%	Medium 199	Lee, T., Wu, R., Brody, A., Barrett, J., and Nettesheim, P.: Growth and Differentiation of Hamster Tracheal Epithelial Cells in Culture, *Exp Lung Res 6*, 27, 1984 (*406*)
	Hamster, Syrian gold, male, 6 weeks- 4 months	Tracheal	Trypsin: 0.25%	PBS with EDTA	Goldman, W.E., Baseman, J.B.: Selective Isolation and Culture of a Proliferating Epithelial Cell Population from the Hamster Trachea, *In Vitro 16*, 313, 1980 (*506*)
Human	Human	Colonic epithelial	Collagenase: Neutral Protease: 0.3% Deoxyribonuclease I: 0.05%	RPMI 1640	Fukushima, K. and Fiocchi, C.: Paradoxical Decrease of Mitochondrial DNA Deletions in Epithelial Cells of Active Ulcerative Colitis Patients., *Am J Physiol Gastrointest Liver Physiol Vol. 286*, G804-13, 2004 (*10355*)
	Human	Human tracheal epithelium	Protease Type XIV: 0.04%	DMEM/F12	Widdicombe JH, Sachs LA, Morrow JL, and Finkbeiner WE.: Expansion of cultures of human tracheal epithelium with maintenance of differentiated structure and function, *Biotechniques 39(2)*, 249, 2005 (*9824*)
	Human	Epithelial	Collagenase: 2.0%	DMEM/Ham's F-12	Emerman, J. and Wilkinson, D.: Routine Culturing of Normal, Dysplastic and Malignant Human Mammary Epithelial Cells from Small Tissue Samples, *In Vitro Cell Dev Biol 26*, 1186, 1990 (*429*)
	Human	Epithelial	Pronase: 0.1%	PBS	Gruenert, D.C., Basbaum, C.B., and Widdicombe, J.H.: Long-Term Culture of Normal and Cystic Fibrosis Epithelial Cells Grown Under Serum-Free Conditions, *In Vitro Cell Dev Biol 26*, 411, 1990 (*440*)
	Human	Epithelial Sweat gland	Collagenase Type 2: 0.2%	(see reference)	Wood, L. and Neufeld, E.: A Cystic Fibrosis Phenotype in Cells Cultured from Sweat Gland Secretory Coil. Altered Kinetics of 36Cl Efflux, *J Biol Chem 265*, 12796, 1990 (*568*)
	Human, women, 27-49 years	Epithelial Ovary	Trypsin: 0.125%	HBSS, CMF	Auersperg, N., Siemens, C.H., and Myrdal, S.E.: Human Ovarian Surface Epithelium In Primary Culture, *In Vitro 20*, 743, 1984 (*535*)
	Human, infant and neonate	Epithelial Prostate	Trypsin: 0.1%	HBSS	Lechner, J., Babcock, M., Marnell, M., Narayan, K., and Kaighn, M.: Normal Human Prostate Epithelial Cell Cultures, *Methods Cell Biol 21*, 195, 1980 (*631*)
Mouse	Mouse	Submandibular salivary	Collagenase Type 2 or 3: 0.16%	DMEM	Durban, E: Submandibular Salivary Epithelial Cells,*Cell & Tissue Culture: Laboratory Procedures Vol. 1*,Doyle, A., Griffiths, J., and Newell, D., John Wiley and Sons, Ltd., 12B:2.1, 1995 (*1272*)
	Mouse	Esophageal	Trypsin: 0.25%	PBS, CMF	Katayama, M., Kan, M.: Heparin-Binding (Fibroblast) Growth Factors Are Potential Autocrine Regulators of Esophageal Epithelial Cell Proliferation, *In Vitro Cell Dev Biol 27*, 533, 1991 (*467*)
	Mouse, BALB/c, male, 3-5 months	Epithelial Submandibular salivary gland	Collagenase Type 3: 0.16% , 1:1 v/v	DMEM with 15 mM HEPES	Durban, E.M.: Mouse Submandibular Salivary Epithelial Cell Growth and Differentiation in Long-Term Culture:Influence of the Extracellular Matrix, *In Vitro Cell Dev Biol 26*, 33, 1990 (*437*)
	Mouse, BALB/cfC3H or BALB/c 8-12 day mid-pregnant	Epithelial Submandibular gland	Collagenase Type 3: 0.1%	HBSS	Yang, J., Flynn, D., Larson, L., and Hamamoto, S.: Growth in Primary Culture of Mouse Submandibular Epithelial Cells, *In Vitro 18*, 435, 1982 (*520*)
	Mouse BALB/cfC3H	Mammary tumors Epithelial	Collagenase: 1.0%	HBSS	Yang, J., Guzman, R., Richards, J., and Nandi, S.: Primary Cultures of Mouse Mammary Tumor Epithelial Cells Embedded in Collagen Gels, *In Vitro 16*, 502, 1980 (*507*)
	Mouse, C3H, 6-8 weeks	Epithelial	Collagenase: 0.10%	DMEM	Lillehaug, J., Mondal, S., and Heidelberger, C.: Establishment of Epithelial Cell Lines from Mouse Regenerating Liver, *In Vitro 15*, 910, 1979 (*504*)
Porcine	Porcine, 5-60 kg	Retinal pigment epithelial cells	Collagenase: 2%	DMEM	Wiencke, A., Kiilgaard, J., Nicolini, J., Bundgaard, M., Ropke, C., and La Cour, M.: Growth of Cultured Porcine Retinal Pigment Epithelial Cells, *Acta Opthalmol Scand 81(2)*, 170, 2003 (*9825*)
	Porcine	Trachea	Neutral Protease: 0.2%	HBSS	De Buysscher, E., Kennedy, J., and Mendicino, J.: Synthesis of Mucin Glycoproteins by Epithelial Cells Isolated from Swine Trachea by Specific Proteolysis, *In Vitro 20*, 433, 1984 (*534*)
Rabbit	Rabbit, New Zealand white, adult	Colon	Neutral Protease: 0.3%	PBS	Vidrich, A., Racindranath, R., Farsi, K., and Targan, S.: A Method for the Rapid Establishment of Normal Adult Mammalian Colonic Epithelial Cell Cultures, *In Vitro Cell Dev Biol 24 (3)*, 188, 1988 (*918*)
	Rabbit, New Zealand white, male, 4-5lb.	Gastric Parietal and chief	Collagenase Type 2: 0.08%	Sodium phosphate buffer	Chew, C.S., Brown,M.R.: Release of Intracellular Ca2+ and Elevation of Inositol Triphosphate by Secretagogues in Parietal and Chief Cells Isolated from Rabbit Gastric Mucosa, *Biochim Biophys Acta 888*, 116, 1986 (*326*)
	Rabbit, New Zealand white estrous, female, 4-5 months	Mesothelial and surface epithelial Ovaries	Trypsin: 0.125%-0.5%	Medium 199	Nicosia, S., Johnson, J., and Streibel, E.: Isolation and Ultrastructure of Rabbit Ovarian Mesothelium(Surface Epithelium), *Int J Gynecol Pathol 3*, 348, 1984 (*542*)
	Rabbit, fetal	Epithelial Gastric	Collagenase Type 3: 0.10%	HBSS	Logsdon, C.D., Bisbee, C.A., Rutten, M.J. and Machen, T.E.: Fetal Rabbit Gastric Epithelial Cells Cultured on Floating Collagen Gel, *In Vitro 18*, 233, 1981 (*517*)
Rat	Rat, SD, male, 150 - 250 g	Epithelial Stomach	Pronase: 0.15%	Medium 199	Dial, E., Kao, Y., and Lichtenberger, L.: Effects of 16,16-Dimethyl Prostaglandin E2 On Glycoprotein And Lipid Synthesis of Gastric Epithelial Cells Grown in a Primary Culture, *In Vitro Cell Dev Biol 27*, 39, 1991 (*464*)
	Rat, Wistar, neonatal	Epithelial	Trypsin: 0.1%	HBSS	Jassal, D., Han, R., Caniggia, I., Post, M., and Tanswell, A.: Growth of Distal Fetal Rat Lung Epithelial Cells in a Defined Serum-Free Medium, *In Vitro Cell Dev Biol 27A*, 625, 1991 (*471*)
	Rodent, various (see reference)	Epithelial	Trypsin: 0.2%	MEM, PBS	Robinson, C., and Wu, R.: Culture of Conducting Airway Epithelial Cells in Serum-Free Medium, *J Tiss Cul Meth 13*, 95, 1991 (*1239*)

[CF=Calcium Free • CMF=Calcium Magnesium Free • BSS=Balanced Salt Solution]

Epithelial (Con't)

Epithelial (Con't)

Species		Cell(s)	Enzyme(s)	Medium	Reference
Rat	Rat (ACI/NMs X BUF/Mna) F1, male, 28 months Rat (ACI/MNs) male, 8 weeks	Epthelial	Collagenase Type 3: 0.1%	Eagle's MEM Serum-free	Masuda, A., Ohtsuka, K., and Matsuyama, M.: Establishment of Functional Epithelial Cell Lines from a Rat Thyoma and a Rat Thymus, *In Vitro Cell Dev Biol 26*, 713, 1990 (*448*)
	Rat, Fischer, male, 4-6 weeks	Epithelial Esophagus	Hyaluronidase: 0.1%	HEPES BSS	Babcock, M., Marino, M., Gunning, W., and Stoner, G.: Clonal Growth and Serial Propagation of Rat Esophageal Epithelial Cells, *In Vitro 19*, 403, 1983 (*526*)
	Rat, Fischer, Lewis and SD, male, 10-18 months	Epithelial	Trypsin: 0.05%	HBSS CMF	Herring, A., Raychaudhuri, R., Kelley, S., and Iype, P.: Repeated Establishment of Diploid Epithelial Cell Cultures from Normal and Partially Hepatectomized Rats, *In Vitro 19*, 576, 1983 (*528*)
	Rat, Wistar, 12 day	Epithelial	Trypsin: 0.05%	HBSS, CMF	Malan-Shibley, L., and Iype, P.: Influence of Cultures on Cell Morphology/Tyrosine Aminotransferase Levels, *Exp Cell Res 131*, 363, 1981 (*391*)
	Rat, Fischer, adult, 200-250 g	Epithelial	Hyaluronidase: 0.0075%	KCl-NaCl HEPES Buffer	Williams, G., and Gunn, J.: Long-Term Culture of Adult Rat Liver Epithelial Cells, *Exp Cell Res 89*, 139, 1974 (*405*)
	Rat, Fischer, 10 day	Epithelial-like	Trypsin: 0.25%	PBS	Williams, G., Weisburger, E., and Weisburger, J.: Isolation and Long-Term Cell Culture of Epithelial-Like Cells from Rat Liver, *Exp Cell Res 69*, 106, 1971 (*402*)

Eye

Eye

Species		Cell(s)	Enzyme(s)	Medium	Reference
Bovine	Bovine	Pericyte	Collagenase: 0.2%	DMEM	Bryan, B. and D'Amore, P.: Pericyte Isolation and Use in Endothelial/Pericyte Coculture Models, *Meth Enzymol 443*, 315, 2008
	Bovine	Microvascular endothelial	Collagenase/Dispase 0.1%	MEM	Bowman, P., Betz, A., and Goldstein, G.: Primary Culture of Microvascular Endothelial Cells From Bovine Retina, *In Vitro 18 (7)*, 626, 1982
Human	Human	Corneal stromal stem	Neutral Protease 1.2 u/ml Collagenase: 0.1%	DMEM	Du, Y., Roh, D., Funderburgh, M., Mann, M., Marra, K., Rubin, J., Li, X. and Funderburgh, J.: Adipose-Derived Stem Cells Differentiate to Keratocytes *In Vitro., Mol Vis 16*, 2680, 2010
	Human, 18-68 yr	Corneal endothelial	Collagenase: 0.2% Neutral Protease: 1.0%	DMEM/F12	Li Wei, Sabater Alfonso L, Chen Ying-Ting, Hayashida Yasutaka, Chen Szu-Yu, He Hua, Tseng Scheffer C G: A novel method of isolation, preservation, and expansion of human corneal endothelial cells, *Inv Ophthal Visual Sci 48*, 614-20, 2007 (*10306*)
Rat	Rat, Fisher, adult	Retinal	PDS kit: per instructions	MEM	Suzuki, T., Mandai, M., Akimoto, M., Yoshimura, N. and Takahashi, M.: The Simultaneous Treatment of MMP-2 Stimulants in Retinal Transplantation Enhances Grafted Cell Migration into the Host Retina., *Stem Cells 24*, 2406, 2006 (*10515*)

Heart

Heart

Species		Cell(s)	Enzyme(s)	Medium	Reference
Bovine	Bovine (also rat)	Heart Adrenal chromaffin Paraneurons	Trypsin: 0.06%	25mM HEPES buffered Locke's solution, CMF	Trifaro, J., Tang, R., and Novas, M.: Monolayer Co-Culture of Rat Heart Cells and Bovine Adrenal Chromaffin Paraneurons, *In Vitro Cell Dev Biol 26*, 335, 1990 (*438*)
Canine	Canine, adult	Cardiomyocytes	Collagenase Type 2: 0.05% Protease: 0.008%	M199	Gavi, S., Yin, D., Shumay, E., Wang, H. and Malbon, C.: Insulin-Like Growth Factor-I Provokes Functional Antagonism and Internalization of Beta1-Adrenergic Receptors., *Endocrinology 148*, 2653-62, 2007 (*10505*)
Chicken	Chick, embryonic, 11-12-day-old	Myocytes	Trypsin: 0.025%	CMF solution	Murphy, E., Aiton, J., Russell, C., and Lieberman, M.: Calcium Elevation in Cultured Heart Cells: Its Role in Cell Injury, *Am J Physiol Cell Physiol 245 (14)*, C316, 1983 (*1188*)
Feline	Cat, mongrel, adult, 1.8-2.8 kg	Myocytes Ventricular	Collagenase Type 2: 0.12%	Kreb's Henseleit, CF	Silver, L., Hemwall, E., Marino, T., and Houser, S.: Isolation and Morphology of Calcium-Tolerant Feline Ventricular Myocytes, *Am J Physiol 245*, H891, 1983 (*293*)
Fish	Octopus, E. cirhosa, 260-352 g	Systemic heart cardiomyocytes	Collagenase: 0.025% Trypsin: 0.02%	(see reference)	Altimiras J., Hove-Madsen L., and Gesser H.: Ca(2+) Uptake in the Sarcoplasmic Reticulum from the Systemic Heart of Octopod Cephalopods, *J Exp Biol 202*, 2531, 1999 (*9834*)
Frog	Frog (*Rana esculenta*)	Myocytes	Trypsin: 0.04%	CF Ringer	Arrio-Dupont, M., and de Nay, D.: High Yield Preparation of Calcium-Tolerant Myocytes From Frog Ventricles, *Biol Cell 54*, 164, 1985 (*339*)
Guinea-Pig	Guinea pig, adult	Cardiomyocytes	Collagenase Type 2: 100 u/ml	M-199	Zorn-Pauly K, Schaffer P, Pelzmann B, Bernhart E, Lang P, and Koidl B: L-type and T-type Ca2+ current in cultured ventricular guinea pig myocytes, *Physiol Res 53(4)*, 369, 2004 (*9865*)
Human	Human	Atrial cardiomyocytes	Collagenase Type 1: 286 u/ml Protease: 5 u/ml	see reference	Voigt, N., Makary, S., Nattel, S. and Dobrev, D.: Voltage-Clamp-Based Methods for the Detection of Constitutively Active Acetylcholine-Gated I(K,ACh) Channels in the Diseased Heart., *Methods Enzymol 484*, 653, 2010
	Human	Coronary artery smooth muscle	Collagenase Type 2: 0.1% Elastase: 0.05%	DMEM	Jensen, B., Swigart, P., Laden, M., DeMarco, T., Hoopes, C. and Simpson, P.: The Alpha-1D Is the Predominant Alpha-1-Adrenergic Receptor Subtype in Human Epicardial Coronary Arteries., *J Am Coll Cardiol 54*, 1137, 2009 (*10580*)
Mouse	Mouse	Cardiomyocytes	Collagenase Type 1: 0.17%	PBS	Chen, H., Yong, W., Ren, S., Shen, W., He, Y., Cox, K., Zhu, W., Li, W., Soonpaa, M., Payne, RM, Franco, D., Field, L., Rosen, V., Wang, Y. and Shou, W.: Overexpression of Bone Morphogenetic Protein 10 in Myocardium Disrupts Cardiac Postnatal Hypertrophic Growth., *J Biol Chem 281*, 27481, 2006 (*10354*)
	Mouse	Ventricular myocytes and mesenteric arterial SMC	Collagenase Type 2: 0.06% Papain: 0.175%	Krebs-Ringer	Lu Tong, Ye Dan, Wang Xiaoli, Seubert John M, Graves Joan P, Bradbury J Alyce, Zeldin Darryl C, Lee Hon-Chi: Cardiac and vascular KATP channels in rats are activated by endogenous epoxyeicosatrienoic acids through different mechanisms, *J Physiol 575*, 627-44, 2006 (*10293*)

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Heart (Con't)

Heart (Con't)

Species		Cell(s)	Enzyme(s)	Medium	Reference
Mouse	Mouse, neonatal	Cardiomyoctes	Collagenase Type 1: 150 u/ml Trypsin: 0.01%	M199	Takahashi N, Wang X, Tanabe S, Uramoto H, Jishage K, Uchida S, Sasaki S, Okada Y.: ClC-3-independent sensitivity of apoptosis to Cl- channel blockers in mouse cardiomyocytes, *Cell Physiol Biochem 15*, 263, 2005 (*10033*)
	Mouse, 6-8 week	Vascular smooth muscle	Papain: 10 u/ml Elastase: .005% Collagenase: 0.05% Deoxyribonuclease I: 1000 u/ml PDS kit: with modifications	EBSS	Qian, Q., Hunter, L., Li, M., Marin-Padilla, M., Prakash, Y., Somlo, S., Harris, P., Torres, V., and Sieck, G.: Pkd2 Haploinsufficiency Alters Intracellular Calcium Regulation in Vascular Smooth Muscle Cells, *Hum Mol Genet 12(15)*, 1875, 2003 (*9846*)
Rabbit	Rabbit	Smooth muscle cells	Collagenase Type 2: 300 u/ml Elastase: 5 u/ml	F10 Ham	Verheye, S., Martinet, W., Kockx, M., Knaapen, M., Salu, K., Timmermans, J., Ellis, J., Kilpatrick, D., DeMayer, G.: Selective Clearance of Macrophages in Atherosclerotic Plaques by Autophagy, *J Am Coll Cardiol 49*, 706, 2007 (*10335*)
Rat	Rat, SD, male	Ventricular myocytes	Collagenase: 0.13% Hyaluronidase: 0.06%	DMEM	Stagg Mark A, Coppen Steven R, Suzuki Ken, Varela-Carver Anabel, Lee Joon, Brand Nigel J, Fukushima Satsuki, Yacoub Magdi H, Terracciano Cesare M: Evaluation of frequency, type, and function of gap junctions between skeletal myoblasts overexpressing connexin43 and cardiomyocytes: relevance to cell transplantation, *FASEB J 20*, 744-6, 2006 (*10275*)
	Rat, SD, 200-250 g	Ventricular myocytes and mesenteric arterial SMC	Collagenase Type 2: 0.06% Papain: 0.175%	Krebs-Ringer	Lu Tong, Ye Dan, Wang Xiaoli, Seubert John M, Graves Joan P, Bradbury J Alyce, Zeldin Darryl C, Lee Hon-Chi: Cardiac and vascular KATP channels in rats are activated by endogenous epoxyeicosatrienoic acids through different mechanisms, *J Physiol 575*, 627-44, 2006 (*10293*)
	Rat, neonatal, adult, 17 day	Ventricular cardiomyocytes	Collagenase Type 2: 0.7-1%	DMEM	Lam ML, Bartoli M, and Claycomb WC.: The 21-day postnatal rat ventricular cardiac muscle cell in culture as an experimental model to study adult cardiomyocyte gene expression, *Mol Cell Biochem 229*, 51, 2002 (*9842*)

Intestine

Intestine

Species		Cell(s)	Enzyme(s)	Medium	Reference
Human	Human	Intestinal epithelial	Collagenase Type 4: 72.5 u/ml	HBSS	Fahlgren, A., Hammarstrom, S., Danielsson, A. and Hammarstrom, M.: Increased Expression of Antimicrobial Peptides and Lysozyme in Colonic Epithelial Cells of Patients with Ulcerative Colitis., *Clin Exp Immunol 131*, 90, 2003 (*10358*)
	Human	Lamina propria mononuclear cells	Collagenase: 0.02%	HBSS	Kanai Takanori, Totsuka Teruji, Uraushihara Koji, Makita Shin, Nakamura Tetsuya, Koganei Kazutaka, Fukushima Tsuneo, Akiba Hisaya, Yagita Hideo, Okumura Ko, Machida Utako, Iwai Hideyuki, Azuma Miyuki, Chen Lieping, Watanabe Mamoru: Blockade of B7-H1 suppresses the development of chronic intestinal inflammation, *J Immunol 171*, 4156-63, 2003 (*10232*)
	Human	Mucosal mononuclear cells	Collagenase Type 3: 0.01% Deoxyribonuclease I: 0.01% Soybean Trypsin Inhibitor: 0.01%	RPMI	Stallmach A, Schaffer F, Hoffmann S, Weber S, Muller-Molaian I, Schneider T, Kohne G, Ecker KW, Feifel G, Zeitz M: Increased state of activation of CD4 positive T cells and elevated interferon gamma production in pouchitis, *Gut 43*, 499-505, 1998 (*10240*)
Mouse	Mouse, 7 week	Interstitial cells	Collagenase: 0.04% Trypsin: 0.02%	(see reference)	Goto Kazunori, Matsuoka Satoshi, Noma Akinori: Two types of spontaneous depolarizations in the interstitial cells freshly prepared from the murine small intestine, *J Physiol 559*, 411-22, 2004 (*10145*)
	Mouse, 0-15 day	Interstitial cells of Cajal	Collagenase Type 2: 0.13%	Hanks	Lee Young Mee, Kim Byung Joo, Kim Hyun Jin, Yang Dong Ki, Zhu Mei Hong, Lee Kyu Pil, So Insuk, Kim Ki Whan: TRPC5 as a candidate for the nonselective cation channel activated by muscarinic stimulation in murine stomach, *Am J Physiol/Gastro 284*, G604-16, 2003 (*10233*)
	Mouse	Lamina propria lymphocytes	Collagenase Type 4: 300 u/ml	PBS	Wu Y, Wang X, Csencsits KL, Haddad A, Walters N, Pascual DW: M cell-targeted DNA vaccination, *Proc Natl Acad Sci U S A 98*, 9318-23, 2001 (*10271*)

Kidney

Kidney

Species		Cell(s)	Enzyme(s)	Medium	Reference
Bovine	Bovine	Papillary duct	Hyaluronidase: 0.2%	Kreb's buffer HEPES buffered saline	Husted, R., Hayashi, M., and Stokes, J.: Characteristics of Papillary Collecting Duct Cells in Primary Culture, *Am J Physiol 255*, F1160, 1988 (*298*)
Canine	Dog, 1-5 months	Proximal tubules	Collagenase Type 1: 0.15%	Krebs Ringer bicarbonate buffer	States, B., Reynolds, R., Lee, J., and Segal, S.: Cystine Uptake By Cultured Cells Originating From Dog Proximal Tubule Segments, *In Vitro Cell Dev Biol 26*, 105, 1990 (*426*)
	Dog, mongrel, 20 Kg	Proximal tubular	Deoxyribonuclease I: 0.0125%	(see reference)	Yau, C., Rao, L., and Silverman, M.: Sugar Uptake Into a Primary Culture of Dog Kidney Proximal Tubular Cells, *Can J Physiol Pharmacol 63*, 417, 1985 (*707*)
Fish	Winter flounder (*Pseudopleur-onectes americanus*) 200 - 500 g	Renal tubule	Trypsin: 0.2%	CMF solution (see reference)	Dickman, K. and Renfro, J.: Primary Culture of Flounder Renal Tubule Cells:Transepithelial Transport, *Am J Physiol 251*, 424, 1986 (*297*)
Guinea-Pig	Guinea-pig, male, 100 g	Single cells	Trypsin: 0.25%	CF salt solution	Phillips, H.: Dissociation of Single Cells from Lung or Kidney Tissue with Elastase, *In Vitro 8*, 101, 1972 (*538*)
Hamster	Hamster (also rat, SD and Wistar, 150-225 g, rabbit, bovine)	Papillary duct	Hyaluronidase: 0.2%	Kreb's buffer HEPES buffered saline	Husted, R., Hayashi, M., and Stokes, J.: Characteristics of Papillary Collecting Duct Cells in Primary Culture, *Am J Physiol 255*, F1160, 1988 (*298*)
Human	Human	Renal cortex	Trypsin: 0.1%	Tissue Culture Grade Water	McAteer, J, Kempson, S., and Evan, A: Culture of Human Renal Cortex Epithelial Cells, *J Tiss Cul Meth 13*, 143, 1991 (*1266*)
	Human	Papillary duct	Collagenase: 400 u/ml	Eagle's MEM-HEPES buffer w/L-glutamine	Trifillis, A. and Kahng, M.: Characterization of an In Vitro System of Human Renal Papillary Collecting Duct Cells, *In Vitro Cell Dev Biol 26*, 441, 1990 (*441*)

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Kidney (Con't)

Kidney (Con't)

Species		Cell(s)	Enzyme(s)	Medium	Reference
Human	Human	Mesangial	Trypsin: 0.25%	DMEM/Ham's F-12	Heieren, M., van der Woude, F., and Balfour Jr., H.: Cytomegalovirus Replicates Efficiently in Human Kidney Mesangial Cells, *Proc Natl Acad Sci U S A 85*, 1642, 1988 (*657*)
Monkey	Monkey	Kidney	Trypsin: 0.01% - 0.00001%	Eagle's MEM	Melnick, J., and Wallis, C.: Problems Related to the Use of Serum and Trypsin in the Growth of Monkey Kidney Cells, *Dev Biol 37*, 77, 1976 (*706*)
Mouse	Mouse, adult	Cortex, proximal tubule	Collagenase: 0.15%	DMEM	Syal Ashu, Schiavi Susan, Chakravarty Sumana, Dwarakanath Vangipuram, Quigley Raymond, Baum Michel: Fibroblast growth factor-23 increases mouse PGE2 production in vivo and in vitro, *Am J Physiol/ Renal 290*, F450-5, 2006 (*10281*)
	Mouse, naive SJL (H-2S)	Proximal tubular epithelial	Deoxyribonuclease I: 15 µg/ml	RPMI 1640	Haverty, T., Kelly, C., Hines, W., Amenta, P., Watanabe, M., Harper, R., Kefalides, N., and Neilson, E.: Characterization of a Renal Tubular Epithelial Cell Line Which Secretes the Autologous Target Antigen of Autoimmune Experimental Interstitial Nephritis, *J Cell Biol 107*, 1359, 1988 (*578*)
Rabbit	Rabbit, New Zealand white, male, 1.5 - 2.0 Kg	Duct	Soybean Trypsin Inhibitor: 0.025%	Hank's Solution with calcium and HEPES	Naray-Fejes-Toth, A., and Fejes-Toth, G.: Immunoelection and Culture of Cortical Collecting Duct Cells, *J Tiss Cul Meth 13*, 179, 1991 (*1267*)
	Rabbit, New Zealand white, female, 2-3 Kg	Renal proximal tubules	Deoxyribonuclease I: 70 u/ml	Modified DME-F12	Rodeheaver, D., Aleo, M., and Schnellmann, R.: Differences in Enzymatic and Mechanical Isolated Rabbit Renal Proximal Tubules: Comparison in Long-Term Incubation, *In Vitro Cell Dev Biol 26*, 898, 1990 (*454*)
	Rabbit (also rat, SD and Wistar, 150-225 g, hamster, bovine)	Papillary duct	Hyaluronidase: 0.2%	Kreb's buffer HEPES buffered saline	Husted, R., Hayashi, M., and Stokes, J.: Characteristics of Papillary Collecting Duct Cells in Primary Culture, *Am J Physiol 255*, F1160, 1988 (*298*)
Rat	Rat, Wistar, 300g	Renal proximal tubules	Collagenase Type 2: 0.2%	DMEM/F12	Deng Aihua, Miracle Cynthia M, Lortie Mark, Satriano Joseph, Gabbai Francis B, Munger Karen A, Thomson Scott C, Blantz Roland C: Kidney oxygen consumption, carbonic anhydrase, and proton secretion, *Am J Physiol/Renal 290*, F1009-15, 2006 (*10305*)
	Rat, male, 150-200 g	Epithelial Proximal tubule	Protease: 0.1%	HBSS/CMF	Elliget, K., and Trump, B.: Primary Cultures of Normal Rat Kidney Proximal Tubule Epithelial Cells for Studies of Renal Cell Injury, *In Vitro Cell Dev Biol 27*, 739, 1991 (*476*)
	Rat, adult	Inner medullary collecting duct Papillae	Collagenase Type 2: 0.1%	PBS	Brion, L., Schwartz, J., Lachman, H., Zavilowitz, B., and Schwartz, G.: Development of H+ Secretion by Cultured Renal Inner Medullary Collecting Duct Cells, *Am J Physiol 257*, F486, 1989 (*300*)
	Rat, Wistar, female, 100-150 g	Glomerular mesangial	Collagenase: 0.025%	RPMI 1640	Wang, J., Kester, M., and Dunn, M.: The Effects of Endotoxin on Platelet-Activating Factor Synthesis in Cultured Rat Glomerular Mesangila Cells, *Biochim Biophys Acta 969*, 217, 1988 (*338*)
	Rat, Wistar, male, 150 - 200 g	Renal target	Collagenase: 1.0% (also 0.7%)	Eagle's MEM	Barlet-Bas, C., Khadouri, C., Marsey, S., and Doucet, A.: Sodium-Independent *In Vitro* Induction of Na+,K+-ATPase by Aldosterone in Renal Target Cells: Permissive Effect of Triiodothyronine, *Proc Natl Acad Sci U S A 85*, 1707, 1988 (*658*)
	Rat,Wistar, 300-400 g	Proximal tubules	Collagenase: 0.15 %	Krebs Henseleit solution	Vinay, P., Gougoux, A., and Lemieux, G.: Isolation of a Pure Suspension of Rat Proximal Tubules, *Am J Physiol 241*, F403, 1981 (*289*)
	Rat	Fibroblasts Kidney	Trypsin: up to 0.25%	Dulbecco-Vogt MEM	Wallach, D., Anderson, W., and Pastan, I.: Activation of Adenylate Cyclase in Cultured Fibroblasts by Trypsin, *J Biol Chem 253*, 24, 1978 (*553*)

Liver

Liver

Species		Cell(s)	Enzyme(s)	Medium	Reference
Avian	Ducklings, 7 day	Hepatocytes	Collagenase: 0.05% Hyaluronidase: 0.05%	DMEM/ F12	Lee J., Culvenor J., Angus P., Smallwood R., Nicoll A., and Locarnini S.: Duck Hepatitis B Virus Replication in Primary Bile Duct Epithelial Cells, *J Virol 75(16)*, 7651, 2001 (*9883*)
Canine	Dog, 13 years old (also rat, guinea-pig, rabbit, human)	Hepatocytes	Collagenase: 90 u/ml	CF EGTA perfusate	Reese, J. and Byard, J.: Isolation And Culture of Adult Hepatocytes from Liver Biopsies, *In Vitro 17*, 935, 1981 (*412*)
	Dog, adult	Hepatocytes	Trypsin: 0.1%	HBSS, CMF	Vickrey, H., Ramon, J., and McCann, D.: Continuous Culture of Normal Adult Mammalian Hepatocytes Exhibiting Parenchymal Functions, *In Vitro 15*, 120, 1979 (*502*)
Chicken	Chicken, adult	Hepatocytes	Collagenase: 0.02%	HEPES, CF	Fraslin, J., Touquette, L., Douaire, M., Menezo, Y., Guillemot, J., and Mallard, J.: Isolation and Long Term Maintenance of Differentiated Adult Chicken Hepatocytes in Primary Culture, *In Vitro Cell Dev Biol 28*, 615, 1992 (*486*)
	Chicken (also rat, SD, 150-250 g)	Hepatocytes	Collagenase Type 4: 6000 units	Medium A	Roseman, S., Weigel, P., Schnaar, R., Kuhlenschmidt, M., Schmell, E., Lee, R., and Lee, Y.: Adhesion of Hepatocytes to Immobilized Sugars. A Threshold Phenomenon, *J Cell Biol 254*, 10830, 1979 (*582*)
	Chicken, Leghorn, white, 10-15 day	Hepatocytes	Deoxyribonuclease I: 0.00125%	PBS	Tarlow, D., Watkins, P., Reed, R., Miller, R., Zwergel, E., and Lane, M.: Lipogenesis and the Synthesis and Secretion of Very Low Density Lipoprotein by Avian Liver Cells in Nonproliferating Monolayer Culture, *J Cell Biol 73*, 332, 1974 (*590*)
	Chick embryos, 5 day	Heart Liver	Trypsin: 3.0%	Tyrode's solution, CMF	Steinberg, M.: "ECM": Its Nature, Origin, And Function in Cell Aggregation, *Exp Cell Res 30*, 257, 1963 (*396*)
	Chick embryonic	Various tissues (heart, liver, skeletal, cardiac)	Trypsin: various grades	CMF Tyrode's solution	Rinaldini, L.: An Improved Method for the Isolation and Quantitative Cultivation of Embryonic Cells, *Exp Cell Res 16*, 477, 1959 (*394*)
Fish	Rainbow trout (*Oncorhynchus mykiss*), male & female, 120-600 g	Hepatocytes	Collagenase Type 2: 0.045%	Kreb's-Ringer bicarbonate buffer, CF	Blair, J.B., Miller, M.R., Pack, D., Barnes, R., Teh, S.J. and Hinton, D.E.: Isolated Trout Liver Cells:Establishing Short-Term Primary Cultures Exhibiting Cell-to-Cell Interactions, *In Vitro Cell Dev Biol 26*, 237, 1990 (*434*)
	Trout, male, 100 - 200 g	Hepatocytes	Collagenase Type 2: 0.045%	HBSS with 0.05M HEPES	Lipsky, M., Sheridan, T., Bennett, R., and May, E.: Comparison of Trout Hepatocyte Culture on Different Substrates, *In Vitro Cell Dev Biol 22*, 360, 1986 (*418*)

[CF=Calcium Free • CMF=Calcium Magnesium Free • BSS=Balanced Salt Solution]

Liver (Con't)

Species		Cell(s)	Enzyme(s)	Medium	Reference
	Trout 100g - 5kg	Hepatocytes	Hyaluronidase: 0.08%	(see reference)	Bailey, G., Taylor, M., and Selivonchick, D.: Aflatoxin B1 Metabolism and DNA Binding in Isolated Hepatocytes From Rainbow Trout *Salmo gairdner*, *Carcinogenesis 3*, 511, 1982 (*1264*)
	Trout (*Salmo gairdneri*), male, 150-200 g Catfish (*Ictalurus punctatus*), male, 100 - 150 g	Hepatocytes	Collagenase: 100u/ml	HBSS	Klaunig, J.: Establishment of Fish Hepatocyte Cultures For Use in *In Vitro* Carcinogenicity Studies, *Natl Cancer Inst Monogr 65*, 163, 1981 (*703*)
Guinea-Pig	Guinea-pig, Hartley, male, 250-300 g	Hepatocytes	Collagenase Type 2:	Kreb's Ringer bicarbonate buffer	Arinze, I. and Kawai, Y.: Adrenergic Regulation of Glycogenolysis in Isolated Guinea-Pig Hepatocytes: Evidence that *B_2-Receptors Mediate Catecholamine Stimulation of Glycogenolysis*, *Arch Biochem Biophys 225*, 196, 1983 (*304*)
	Guinea-pig, young	Hepatocytes	Collagenase: 90 u/ml	CF EGTA perfusate	Reese, J. and Byard, J.: Isolation And Culture of Adult Hepatocytes from Liver Biopsies, *In Vitro 17*, 935, 1981 (*412*)
Human	Human	Hepatocytes	Collagenase: 0.05%	EBSS	Hughes, R., Mitry, R., Dhawan, A., Lehec, S., Girlanda, R., Rela, M., Heaton, N., and Muiesan, P.: Isolation of Hepatocytes from Livers from Non-Heart-Beating Donors for Cell Transplantation, *Liver Transpl 12*, 713, 2006 (*10205*)
	Human	Hepatocytes	Collagenase: 0.05%	HEPES buffer (see reference)	Gomez-Lechon, M., Lopez, P., Donato, T., Montoya, A., Larrauri, A., Gimenez, P., Trullenque, R., Fabra, R., and Castell, J.: Culture of Human Hepatocytes From Small Surgical Liver Biopsies: Biochemical Characterization And Comparison With *in vivo*, *In Vitro Cell Dev Biol 26*, 67, 1990 (*445*)
	Human, male, 17-40 yrs.	Hepatocytes	Collagenase: 0.05%	HEPES buffer	Begue, J., Baffet, G., Campion, J., and Guillouzo, A.: Differential Response of Primary Cultures of Human and Rat Hepatocytes to Aflatoxin B1-Induced Cytotoxicity and Protection by the Hepatoprotective agent(+)-Cyanidanol-3, *Biol Cell 63*, 327, 1988 (*341*)
	Human, 51-75 years old (also rat, guinea-pig, rabbit, dog)	Hepatocytes	Collagenase: 90 u/ml	CF EGTA perfusate	Reese, J. and Byard, J.: Isolation And Culture of Adult Hepatocytes from Liver Biopsies, *In Vitro 17*, 935, 1981 (*412*)
	Human	Hepatocytes	Trypsin: 0.1%	HBSS, CMF	Kaighn, M.: Human Liver Cells,*Tissue Culture Methods / Applications*,(Kruse, P., Patterson, M. eds), 54, 1973 (*702*)
Monkey	Monkey (*Macaca fascicularis*), adult, 5-6 Kg	Hepatocytes	Trypsin: 160 u/ml	HEPES buffer	Ulrich, R., Aspar, D., Cramer, C., Kletzien, R., and Ginsberg, L.: Isolation and Culture of Hepatocytes from the Cynomolgus Monkey (*Macaca fascicularis*), *In Vitro Cell Dev Biol 26*, 815, 1990 (*452*)
Mouse	Mouse	Liver epithelial progenitor cells	Collagenase Type 4: 0.1% Deoxyribonuclease I: 0.05%	DMEM	Li Wen-Lin, Su Juan, Yao Yu-Cheng, Tao Xin-Rong, Yan Yong-Bi, Yu Hong-Yu, Wang Xin-Min, Li Jian-Xiu, Yang Yong-Ji, Lau Joseph T Y, Hu Yi-Ping: Isolation and characterization of bipotent liver progenitor cells from adult mouse, *Stem Cells 24*, 322-32, 2006 (*10248*)
	Mouse, C3H, 6-8 weeks	Epithelial	Collagenase: 0.10%	DMEM	Lillehaug, J., Mondal, S., and Heidelberger, C.: Establishment of Epithelial Cell Lines from Mouse Regenerating Liver, *In Vitro 15*, 910, 1979 (*504*)
	Mouse, 20-30 g	Parenchymal and non-parenchymal	Hyaluronidase: 0.1%	Hank's w/ Insulin, CMF	Crisp, D., and Pogson, C.: Glycolytic and Gluconeogenic Enzyme Activities in Parenchymal and Non-parenchymal Cells from Mouse Liver, *Biochem J 126*, 1009, 1972 (*309*)
Porcine	Porcine, male, 2-3 week	Hepatocytes	Collagenase: 0.05%	DMEM	Raman Priya, Donkin Shawn S, Spurlock Michael E: Regulation of hepatic glucose metabolism by leptin in pig and rat primary hepatocyte cultures, *Am J Physiol Regul Integr Comp Physiol 286*, R206-16, 2004 (*10268*)
Rabbit	Rabbit, New Zealand white	Hepatocytes	Collagenase: 90 u/ml	CF EGTA perfusate	Reese, J. and Byard, J.: Isolation And Culture of Adult Hepatocytes from Liver Biopsies, *In Vitro 17*, 935, 1981 (*412*)
Rat	Rat, Fisher, E14	Hepatocytes	Collagenase Type 1: 0.22%	HBSS	Isabel Zvibel, Miri Bronstein, Einav Hubel, Ella Bar-Lev, Zamir Halpern, Ran Oren: Isolation, characterization and culture of Thy1-positive cells from fetal rat livers, *World J Gastroenterol 12*, 3841-7, 2006 (*10303*)
	Rat, 200-300 g	Hepatocytes	Collagenase Type 1: 0.067%	HBSS	Mula, N., Cubero, F., Codesal, J., de Andres, S., Escudero, C., Garcia-Barrutia, S., Millan, I., Arahuetes, R. and Maganto, P.: Survival of Allogeneic Hepatocytes Transplanted into the Thymus., *Cells Tissues Organs 188*, 270, 2008 (*10371*)
	Rat, SD, 8 - 12 day	Parenchymal hepatocytes	Collagenase Type 4: 80 u/ml	HBSS	Davila, J., Reddy, C., Davis, P. and Acosta, D.: Toxicity Assessment of Papaverine Hydrochloride and Papaverine-Derived Metabolites in Primary Cultures of Rat Hepatocytes, *In Vitro Cell Dev Biol 26*, 515, 1990 (*444*)
	Rat, SD, male, 250-350 g	Hepatocytes	Collagenase Type 2: 0.05%	HBSS, CMF	Liu, J., Kershaw, W., and Klaassen, C.: Rat Primary Hepatocyte Cultures are a Good Model for Examining Metallothionein-Induced Tolerance to Cadmium Toxicity, *In Vitro Cell Dev Biol 26*, 75, 1990 (*450*)
	Rat, Wistar, male, 200 g	Parenchymal	Collagenase Type 1:	HEPES, modified	Kindberg, G., Gudmundsen, O., and Berg, T.: The Effect of Vandate on Receptor-mediated Endocytosis of Asialoorosomucoid in Rat Liver Parenchymal Cells, *J Biol Chem 265*, 8999, 1990 (*572*)
	Rat, SD, male, 175-225 g	Hepatocytes	Collagenase:	Kreb's Ringer bicarbonate buffer	Reddy, S., Amick, G., Cooper, R., and Damun, Z.: Insulin Stimulates the Activity of a Protamine in Isolated Rat Hepatocytes, *J Biol Chem 265*, 7748, 1990 (*570*)
	Rat, SD, neonatal, 8 - 10 days	Hepatocytes	Collagenase Type 4: 0.05%	Hanks' BSS, CF	Davila, J., Lenherr, A., and Acosta, D.: Protective Effect of Flavonoids on Drug-Induced Hepatotoxicity *In Vitro*, *Toxicology 57*, 267, 1989 (*673*)
	Rat, Fischer 344, male, 14 months	Hepatocytes	Collagenase: 0.05%	Ringer's biocarbonate buffer	Nagy, I., Ohno-Iwashita, Y., Ohta, M., Nagy, V., Kitani, K., Ando, S., and Imahori, K.: Effect of Perfringolysin O on the Lateral Diffusion Constant of Membrane Proteins of Hepatocytes as Revealed by Fluorescence Recovery After Photobleaching, *Biochim Biophys Acta 939*, 551, 1988 (*327*)
	Rat, SD, male, 200-300 g	Hepatocytes	Collagenase: 0.05%	HEPES	Voss, A. and Sprecher, H.: Metabolism of 6,9,12-Octadecatetraenoic and 6,9,12,15-Octadecatetraenoic Acid, *Biochim Biophys Acta 958*, 153, 1988 (*328*)
	Rat, Wistar, male,3 month, 200 g	Parenchymal Endothelial Kupffer	Collagenase Type 1: 0.05%	Krebs Henseleit	Kuiper, J., Zijlstra, F., Kamps, J, and vanBerkel, T.: Identification of Prostaglandin D2 as the Major Eiconsanoid from Liver Endothelial and Kupffer Cells, *Biochim Biophys Acta 959*, 143, 1988 (*329*)

[CF=Calcium Free • CMF=Calcium Magnesium Free • BSS=Balanced Salt Solution]

Species		Cell(s)	Enzyme(s)	Medium	Reference
Rat	Rat, Wistar, male, 200 - 300 g	Parenchymal Kupffer	Collagenase Type 2: 0.05%	HBSS with $CaCl_2$	Cai, H., He, Z., and Ding, Y.: Effects of Monocyte Macrophages Stimulation on Hepatic Lipoprotein Receptors, *Biochim Biophys Acta 958*, 334, 1988 (*331*)
	Rat, Wistar, female, 17 days	Hepatocytes	Collagenase: 0.05%	HBSS	Cotariu, D., Barr-Nea, L., Papo, N., and Zaidman, J.: Induction of gamma-Glutamyl Transferase by Dexamethasone in Cultured Rat Hepatocytes, *Enzyme 40*, 212, 1988 (*386*)
	Rat, SD, male, 350 - 450 g	Lipocytes Kupffer Sinusoidal endothelial	Collagenase: 0.015%	DMEM/Ham's F-12	Friedman, S. and Roll, F.: Isolation and Culture of Hepatic Lipocytes,Kupffer Cells, and Sinusoidal Endothelial Cells by Density Gradient Centrifugation with Stractan, *Anal Biochem 161*, 207, 1987 (*301*)
	Rat, SD, male	Hepatocytes	Collagenase: 100 - 200 µg/g body weight	Eagle's Eagle's w/HEPES HBSS	Oka, J. and Weigel, P.: Monensin Inhibits Ligand Dissociation Only Transiently and Partially and distinguishes two galactosyl receptor pathways in isolated rat hepatocytes, *J Cell Physiol 133*, 243, 1987 (*595*)
	Rat, SD, female, 100-180 g	Hepatocytes	Collagenase: 0.5%	Krebs Ringer bicarbonate buffer	Schwarz, K., Lanier, S., Carter, E., Homcy, C., and Graham, R.: Rapid Reciprocal Changes in Adrenergic Receptors in Intact Isolated Hepatocytes During Primary Cell Culture, *Mol Pharmacol 27*, 200, 1985 (*639*)
	Rat, SD, male, 270 - 320 g	Hepatocytes	Collagenase: 0.04%	Bicarbonate buffer with calcium added	Brass, E., Garrity, M., and Robertson, R.: Inhibition of Glucagon-Stimulated Hepatic Glycogenolysis by E-Series Prostaglandins, *FEBS Lett 169*, 293, 1984 (*410*)
	Rat, Fischer, Lewis and SD, male 10-18 months	Epithelial	Trypsin: 0.05%	HBSS CMF	Herring, A., Raychaudhuri, R., Kelley, S., and Iybe, P.: Repeated Establishment of Diploid Epithelial Cell Cultures from Normal and Partially Hepatectomized Rats, *In Vitro 19*, 576, 1983 (*528*)
	Rat, Wistar, male, 3 month old	Endothelial Kupffer Parenchymal	Collagenase: 0.05%	HBSS	Nagelkenke, J., Barto, K., and Berkel, T.: In Vivo and in Vitro Uptake and Degradation of Acetylated Low Density Lipoprotein by Rat Liver Endothelial, Kupffer, and Parenchymal Cells, *J Biol Chem 258 (20)*, 12221, 1983 (*940*)
	Rat, SD, male/female, 250-300 g	Hepatocytes	Collagenase: 100 u/ml	Krebs Henseleit bicarbonate buffer	Studer, R. and Borle, A.: Differences between Male and Female Rats in the Regulation of Hepatic Glycogenolysis. The Relative Role of Calcium and cAMP in Phosphorylase Activation by Catecholamines, *J Biol Chem 257*, 7987, 1982 (*556*)
	Rat, Wistar, 12 day	Epithelial	Trypsin: 0.05%	HBSS, CMF	Malan-Shibley, L., and Iype, P.: Influence of Cultures on Cell Morphology/Tyrosine Aminotransferase Levels, *Exp Cell Res 131*, 363, 1981 (*391*)
	Rat, SD	Hepatocytes	Collagenase: 90 u/ml	CF EGTA perfusate	Reese, J. and Byard, J.: Isolation And Culture of Adult Hepatocytes from Liver Biopsies, *In Vitro 17*, 935, 1981 (*412*)
	Rat, Wistar, female, fetus	Hepatocytes	Collagenase: 0.025%	HEPES buffer	Gugen-Guillouzo, C., Tichonicky, L., Szajnert, M., and Kruh, J.: Changes in Some Chromatin and Cytoplastic Enzymes of Perinatal Rat Hepatocytes, *In Vitro 16*, 1, 1980 (*505*)
	Rat, SD, male, 250-300 g	Parenchymal	Collagenase Type 2: 0.05%	Kreb's Henseleit bicarbonate buffer	Yamada, S., Otto, P., Kennedy, D., and Whayne, T.: The Effects of Dexamethasone on Metabolic Activity of Hepatocytes in Primary Monolayer Culture, *In Vitro 16*, 559, 1980 (*508*)
	Rat, SD, 7-10 day	Liver	Collagenase: 0.05%	HBSS modified (see reference)	Acosta, D., Anuforo, D., and Smith, R: , *J Tiss Cul Meth 6*, 35, 1980 (*1268*)
	Rat, Wistar, male, 200-250 g	Hepatocytes	Hyaluronidase: 460 u/ml	Saline	Poli, G., Gravela, E., Albano, E., and Dianzani, M.: Studies on Fatty Liver with Isolated Hepatocytes: II., The Action of Carbon Tetrachloride on Lipid Peroxidation, Protein and Triglyceride Synthesis and Secretion, *Exp Mol Pathol 30*, 116, 1979 (*408*)
	Rat (also chicken)	Hepatocytes	Collagenase Type 3 & 4:	HEPES	Weigel, P., Schnaar, R., Kuhlenschmidt, M., Schmell, E., Lee, R., Lee, Y., and Roseman, S.: Adhesion of Hepatocytes to Immobilized Sugars, *J Biol Chem 254 (21)*, 10830, 1979 (*1032*)
	Rat, SD, male, 200 g	Hepatocytes	Collagenase Type 1: 100 u/ml	Buffers 1 & 2 (see reference)	Rubin, K., Kjellen, L., and Oslashbrink, B.: Intercellular Adhesion between Juvenile Liver Cells.. A Method to Measure the Formation of Stable Lateral Contacts Between Cells Attached to a Collagen Gel, *Exp Cell Res 109*, 413, 1977 (*387*)
	Rat (WAG/RIJ), female, 24, 30, and 37 months	Parenchymal	Collagenase Type 1: 0.05% - 0.06%	HEPES buffer	VanBezodijen, C., Grell, T., and Knook, D.: Effect of Age on Protein Synthesis by Isolated Liver Parenchymal Cells, *Mech Ageing Dev 6*, 293, 1977 (*630*)
	Rat, Fisher, adult, male	Hepatocytes	Collagenase Type 1: 100 u/ml	HBSS (see reference)	Williams, G., Bermudez, E., and Scaramuzzino, D.: Rat Hepatocytes Primary Cell Cultures III. Improved Dissociation and Attachment Techniques and the Enchancement of Survival by Culture Medium, *In Vitro 13 (12)*, 809, 1977 (*826*)
	Rat, Fischer, male, adult, 170-265 g	Hepatocytes	Collagenase: 0.05%-0.10%	Williams E	Laishes, B., and Williams, G.: Conditions Affecting Primary Cell Cultures of Functional Adult Rat Hepatocytes. I.. The Effect of Insulin, *In Vitro 12*, 521, 1976 (*496*)
	Rat, neonate, 3 day	Hepatocytes	Trypsin: 0.25%	PBS, CMF	Bausher, J., and Schaeffer, W.: A Diploid Rat Liver Cell Culture. 1. Characterization and Sensitivity to Aflatoxin B1, *In Vitro 9*, 286, 1974 (*540*)
	Rat, SD, albino, male	Parenchymal	Hyaluronidase: 0.1%	Hank's solution, CMF	Bonney, R., Becker, J., Walker, P., and Potter, V.: Primary Monolayer Cultures of Adult Rat Liver Parenchymal Cells Suitable for Study of the Regulation of Enzyme Synthesis, *In Vitro 9*, 399, 1974 (*541*)
	Rat, embryos, 1-3 days	Hepatocytes	Hyaluronidase: 0.10%	HBSS	Gerschenson, L., Berliner, J., and Davidson, M.: The Isolation and Culture of Liver Cells, *Vol. 32*, 733, 1974 (*635*)
	Rat, SD, adult, male, 200-250 g	Parenchymal	Hyaluronidase: 0.10%	HBSS, CF	Howard, R., Lee, J., and Pesch, L.: The fine structure, potassium content, and respiratory activity of isolated rat liver parenchymal cells prepared by improved enzymatic techniques, *J Cell Biol 57*, 642, 1973 (*586*)
	Rat, SD, adult, male, 180-300 g	Parenchymal	Collagenase Type 1: 0.05%	Hank's solution, CF	Bissell, D., Hammaker, L., and Meyer, U.: Parenchymal Cells from Adult Rat Liver in Nonproliferating Monolayer Culture. I. Functional Studies, *J Cell Biol 59*, 722, 1973 (*588*)
	Rat, Wistar, 200-250 g	Hepatocytes	Hyaluronidase: 0.10%	HBSS, CF	Berg, T., Boman, D., and Seglen, P.O.: Induction of Tryptophan Oxygenase in Primary Rat Liver Cell Suspensions by Glucocorticoid Hormone, *Exp Cell Res 72*, 571, 1972 (*404*)

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Species		Cell(s)	Enzyme(s)	Medium	Reference
Rat	Rat, SD, female, 130-160 g	Hepatocytes	Hyaluronidase: 0.08%	HBSS, CF	Johnson, M., Das, N., Butcher, F., and Fain, J.: The Regulation of Gluconeogenesis in Isolated Rat Liver Cells by Glucagon, Insulin, Dibutyryl Cyclic Adenosine Monophosphate, and Fatty Acids, *J Biol Chem 247*, 3229, 1972 (*550*)
	Rat, Wistar, male, 260-310 g	Hepatocytes	Collagenase: 0.01 - 0.08%	HEPES	Seglen, P.: Preparation of Rat Liver Cells, *Exp Cell Res 74*, 450, 1972 (*840*)
	Rat, Fisher, 10 day	Epithelial-like	Trypsin: 0.25%	PBS	Williams, G., Weisburger, E., and Weisburger, J.: Isolation and Long-Term Cell Culture of Epithelial-Like Cells from Rat Liver, *Exp Cell Res 69*, 106, 1971 (*402*)
	Rat, Wistar, male, 6-8 weeks, 80-160 g	Hepatocytes	Hyaluronidase: 1.0%	HBSS, CMF	Iype, P.: Cultures from Adult Rat Liver Cells. 1.Establishment of Monolayer Cell-Cultures from Normal Lver, *J Cell Physiol 78*, 281, 1971 (*596*)
	Rat, 100-200 g	Hepatocytes	Hyaluronidase: 0.10%	HBSS, CF	Haung, Y., and Ebner, K.: Induction of Tyrosine Aminotransferase in Isolated Liver Cells, *Biochim Biophys Acta 191*, 161, 1969 (*318*)
	Rat, SD, adult, 200-300 g	Parenchymal	Hyaluronidase: 0.10% Collagenase Type 1: 0.05%	HBSS, CF	Berry, M., and Friend, D.: High Yield Preparation of Isolated Rat Liver Parenchymal Cells, *J Cell Biol 43*, 506, 1969 (*583*)

Liver (Con't)

Lung

Lung

Species		Cell(s)	Enzyme(s)	Medium	Reference
Bovine	Bovine	Pulmonary microvessel endothelial	Collagenase Type 2: 1000 u/ml	PBS	Del Vecchio, P., Siflinger-Birnboim, A., Belloni, P., Holleran, L., Lum, H., and Malik, A.: Culture and Characterization of Pulmonary Microvascular Endothelial Cells, *In Vitro Cell Dev Biol 28A*, 711, 1992 (*947*)
Guinea-Pig	Guinea-pig, male, 100 g	Single cells	Trypsin: 0.25%	CF salt solution	Phillips, H.: Dissociation of Single Cells from Lung or Kidney Tissue with Elastase, *In Vitro 8*, 101, 1972 (*538*)
Human	Human fetuses, 80 day (also swine fetuses, 70 day, adult Amer Dutch, 250 day)	Lung	Collagenase: 0.01%	HBSS	Hinz, R., and Syverton, J.: Mammalian Cell Cultures for Study of Influenza Virus. I. Preparation of Monolayer Cultures with Collagenase, *Proc Soc Exp Biol Med 101*, 19, 1959 (*662*)
Mouse	Mouse, 8-12 week	Mononuclear cells	Collagenase Type 1: 300 u/ml Deoxyribonuclease I: 50 u/ml	RPMI 1640 medium	Woolard MD, Hodge LM, Jones HP, Schoeb TR, and Simecka JW: The upper and lower respiratory tracts differ in their requirement of IFN-gamma and IL-4 in controlling respiratory mycoplasma infection and disease, *J Immunol 172*, 6875, 2004 (*10014*)
	Mouse	Alveolar epithelial cells	Neutral Protease: Deoxyribonuclease I: 0.01%	DMEM	Paine R 3rd, Wilcoxen SE, Morris SB, Sartori C, Baleeiro CE, Matthay MA, and Christensen PJ: Transgenic overexpression of granulocyte macrophage-colony stimulating factor in the lung prevents hyperoxic lung injury, *Am J Pathol 163*, 2397, 2003 (*10011*)
	Mouse, 6-8 week	Lung and lymph node cells	Collagenase Type 2: 0.1% Deoxyribonuclease I: 0.002%	RPMI 1640	Vermaelen KY, Carro-Muino I, Lambrecht BN, and Pauwels RA.: Specific migratory dendritic cells rapidly transport antigen from the airways to the thoracic lymph nodes, *J Exp Med 193*, 51, 2001 (*10015*)
Porcine	Swine fetuses (70 day), adult Amer Dutch 250 day (also human fetuses, 80 day)	Lung	Collagenase: 0.01%	HBSS	Hinz, R., and Syverton, J.: Mammalian Cell Cultures for Study of Influenza Virus. I. Preparation of Monolayer Cultures with Collagenase, *Proc Soc Exp Biol Med 101*, 19, 1959 (*662*)
Rabbit	Rabbit, New Zealand white, adult,male	Alveolar type II	Trypsin: 0.0025%	Joklik's MEM	Finkelstein, J., Maniscalco, W., and Shapiro, D.: Properties of Freshly Isolated Type II Alveolar Epithelial Cells, *Biochim Biophys Acta 762*, 398, 1983 (*323*)
	Rabbit, New Zealand, white, male, 1.7 kg	Lung	Pronase: 0.2%	Kreb's serum substitute solution, CMF	Gould, M., Clements, J., Jones, A., and Felts, J.: Dispersal of Rabbit Lung into Individual Viable Cells: A New Model for the Study of Lung Metabolism, *Science 178*, 1209, 1972 (*665*)
Rat	Rat, SD, 250-300g	Alveolar epithelial	Elastase: 3-4.5 u/ml	RPMI 1640	Chen J., Chen Z., Narasaraju T., Jin N., and Liu L.: Isolation of Highly Pure Alveolar Epithelial Type I and Type II Cells from Rat Lungs, *Lab Invest 84*, 727, 2004 (*10006*)
	Rat, SD, 300-400 g	Pulmonary endothelial cells	Collagenase Type 2: 1000 u/ml	DMEM/F-12	King J, Hamil T, Creighton J, Wu S, Bhat P, McDonald F, and Stevens T: Structural and functional characteristics of lung macro- and microvascular endothelial cell phenotypes, *Microvasc Res 67*, 139, 2004 (*10010*)
	Rat, Wistar	Epithelial	Trypsin: 0.1%	HBSS	Jassal, D., Han, R., Caniggia, I., Post, M., and Tanswell, A.: Growth of Distal Fetal Rat Lung Epithelial Cells in a Defined Serum-Free Medium, *In Vitro Cell Dev Biol 27A*, 625, 1991 (*471*)
	Rat, SD, 8 day	Interstitial	Trypsin: 1.125%	HEPES buffer	Berk, J., Franzblau, C., and Goldstein, R.: Recombinant Interleukin-1beta Inhibits Elastin Formation by a Neonatal Rat Lung Fibroblast Subtype, *J Biol Chem 266*, 3192, 1991 (*574*)
	Rat, Wistar, female, virgin	Alveolar epithelial type II	Trypsin: 1%	Eagle's MEM	Fraslon, C., Rolland, G., Bourbon, J., Rieutort, M., and Valenza, C.: Culture of Fetal Alveolar Epithelial Type II Cells in Serum-Free Medium, *In Vitro Cell Dev Biol 27A*, 843, 1991 (*927*)
	Rat, Fischer 344, male	Alveolar type II pneumocytes	Elastase: 40 u/ml	HEPES buffer (see reference)	Mangum, J., Everitt, J., Bonner, J., Moore, L., and Brody, A.: Co-Culture of Primary Pulmonary Cells to Model Alveolar Injury and Translocation of Proteins, *In Vitro Cell Dev Biol 26*, 1135, 1990 (*428*)
	Rat, SD, male, 250-400 g	Alveolar type II	Elastase: 40 u/ml	Phosphate-buffered medium (see reference)	Ma, J., LaCagnin, L., Bowman, L., and Miles, P.: Carbon Tetrachloride Inhibits Synthesis of Pulmonary Surfactant Disaturated Phosphatidylcholines and ATP Production in Alveolar Type II Cells, *Biochim Biophys Acta 1003*, 136, 1989 (*314*)
	Rat, Wistar, adult, male and pregnant female (known gestation)	Alveolar type II	Trypsin: 0.1%	RPMI 1640	Batenburg, J., Otto-Verberne, C., Have-Opbroek, A., and Klazinga, W.: Isolation of Alveolar Type II Cells from Fetal Rat Lung by Differential Adherence in Monolayer Culture, *Biochim Biophys Acta 960*, 441, 1988 (*332*)
	Rat, SD, male, 150 - 200 g	Pneumocytes type II	Trypsin: 0.30%	BSS	Brown, L. and Longmore, W.: Altered Phospholipid Secretion in Type II Pneumocytes Isolated from Streptozotocin-diabetic Rats, *Biochim Biophys Acta 878*, 258, 1986 (*325*)
	Rat, SD, male, 180-200 g	Alveolar type II	Elastase: 4.3 u/ml	HEPES (see reference)	Dobbs, L., Gonzalez R., and Williams, M.: An Improved Method for Isolating Type II Cells in High Yield and Purity, *Am Rev Respir Dis 134*, 141, 1986 (*700*)

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Lung (Con't)

Lung (Con't)

Species		Cell(s)	Enzyme(s)	Medium	Reference
Rat	Rat, SD, male, 150-400 g	Alveolar type II	Elastase: 4 u/ml	Auto-Pow Eagle's modified MEM	Goodman, B., Fleischer, R., and Crandall, E.: Evidence for Active Na+ Transport by Cultured Monolayers of Pulmonary Alveolar Epithelial Cells, *Am J Physiol 245*, C78, 1983 (*292*)
	Rat, SD, male/female, 180-250 g	Alveolar type II	Trypsin: 0.30%	BSS	Mason, R., Williams, M., Greenleaf, R., and Clements, J.: Isolation and Properties of Type II Alveolar Cells from Rat Lung, *Am Rev Respir Dis 115*, 1015, 1977 (*697*)
	Rat, Wistar, pathogen free	Alveolar type II	Trypsin: 0.50%	Earle's MEM	King, R.: Metabolic Fate of the Apoproteins of Pulmonary Surfactant, *Am Rev Respir Dis 115*, 73, 1977 (*699*)
	Rat, fetus, 19 days	Alveolar pneumonocytes, type II	Trypsin: 0.1 %	HBSS, CMF	Douglas, W., and Teel, R.: An Organotypic in Vitro Model System for Studying Pulmonary Surfactant Production by Type II Alveolar Pneumonocytes, *Am Rev Respir Dis 113*, 17, 1976 (*698*)
	Rat, adult	Lung	Collagenase: 0.1%	Moscona saline, CMF	Douglas, W., and Kaighn, M.: Clonal Isolation of Differentiated Rat Lung Cells, *In Vitro 10*, 230, 1974 (*493*)
	Rat, SD, male, 100 g	Alveolar type II	Trypsin: 1.0%	Joklik's medium	Kikkawa, Y., and Yoneda, K.: Type II Epithelial Cell of the Lung. I. Method of Isolation, *Lab Invest 30*, 76, 1974 (*623*)

Lymph nodes

Lymph nodes

Species		Cell(s)	Enzyme(s)	Medium	Reference
Mouse	Mouse	Follicular dentritic	Collagenase Type 4: 0.25% Deoxyribonuclease I: 0.5%	HBSS	Kapasi ZF, Qin D, Kerr WG, Kosco-Vilbois MH, Shultz LD, Tew JG, Szakal AK: Follicular dendritic cell (FDC) precursors in primary lymphoid tissues, *J Immunol 160*, 1078-84, 1998 (*10270*)
	Mouse	Lung draining	Collagenase Type 4: 0.1-0.125%	HBSS	Rayamajhi, M., Redente, E., Condon, T., Gonzalez-Juarrero, M., Riches, D. and Lenz, L.: Non-Surgical Intratracheal Instillation of Mice with Analysis of Lungs and Lung Draining Lymph Nodes by Flow Cytometry., *J Vis Exp 51*, 2702, 2011 (*10661*)

Mammary

Mammary

Species		Cell(s)	Enzyme(s)	Medium	Reference
Bovine	Bovine	Epithelial	Hyaluronidase: 0.005%	HBSS	Gibson, C., Vega, J., Baumrucker, C., Oakley, C., and Welsch, C.: Establishment And Characterization Of Bovine Mammary Epithelial Cell Lines, *In Vitro Cell Dev Biol 27*, 585, 1991 (*469*)
	Bovine	Epithelial	Deoxyribonuclease I: 0.04%	HBSS/Medium 199	Baumrucker, C., Deemer, K., Walsh, R., Riss, T., and Akers, R.: Primary Culture of Bovine Mammary Acini on a Collagen Matrix, *Tissue Cell 20 (4)*, 541, 1988 (*874*)
	Bovine, young, lactating, female	Mammary	Collagenase: 0.30 %	HBSS or EBSS	Anderson, C., and Larson, B.: Comparative Maintenance of Function in Dispersed Cell and Organ Cultures, *Exp Cell Res 61*, 24, 1970 (*399*)
	Bovine, dairy, purebred, (also rat, Holtzmann, albino, white)	Secretory Mammary gland	Collagenase: 0.02 - 0.03%	HBSS or EBSS	Schingoethe, D., Hageman, E., and Larson, B.: Essential Amino Acids for Milk Protein Synthesis in the *In Vitro* Secretory Cell and Stimulation by Elevated Levels, *Biochim Biophys Acta 148*, 469, 1967 (*316*)
Goat	Goat, lactating, 1 month	Mammary gland	Collagenase: 0.02 - 0.03%	HBSS or EBSS	Blanco, A., Rife, U., and Larson, B.: Lactate Dehydrogenase Isozymes during Dedifferentiation in Cultures of Mammary Secretory Cells, *Nature 214*, 1331, 1967 (*640*)
Guinea-Pig	Guinea-pig, pregnant, 4-10 day	Mammary gland	Trypsin NF 1:250: 0.25%	Dulbecco phosphate	Turba, F., and Hilpert, N.: Secretion and Resorption of Proteins by Isolated Mammary Gland Cells. German, *Biochem Z 334*, 501, 1961 (*1282*)
Human	Human	Epithelial	Collagenase: 2.0%	DMEM/Ham's F-12	Emerman, J. and Wilkinson, D.: Routine Culturing of Normal, Dysplastic and Malignant Human Mammary Epithelial Cells from Small Tissue Samples, *In Vitro Cell Dev Biol 26*, 1186, 1990 (*429*)
	Human	Tumor, breast	Neuraminidase: 0.8 u/ml	HBSS	Leung, C., and Shiu, R.: Morphological and Proliferative Characteristics of Human Breast Tumor Cells Cultured on Plastic and in Collagen Matrix, *In Vitro 18*, 476, 1981 (*521*)
	Human	Epithelial	Hyaluronidase: 100 u/ml	DMEM/Ham's F-12	Stampfer, M., Hallowes, R., and Hackett, A.: Growth of Normal Human Mammary Cells in Culture, *In Vitro 16 (5)*, 415, 1980 (*856*)
Mouse	Mouse, 12 week	Mammary epithelial	Collagenase: 0.3% Hyaluronidase: 100 u/ml Trypsin: 0.25% Neutral Protease: 0.5% Deoxyribonuclease I: 0.01%	(see reference)	Taddei Ilaria, Deugnier Marie-Ange, Faraldo Marisa M, Petit Valerie, Bouvard Daniel, Medina Daniel, Fsssler Reinhard, Thiery Jean Paul, Glukhova Marina: Beta1 integrin deletion from the basal compartment of the mammary epithelium affects stem cells, *Nat Cell Biol 10*, 716-22, 2008 (*10320*)
	Mouse BALB/cCrgl, mature	Epithelial	Pronase: 0.01%	Hepes buffered medium 199	Bandyopadhyay, G., Imagawa, W., Wallace, D., and Nandi, S.: Proliferative effects of insulin and epidermal growth factor on mouse mammary epithelial cells in primary culture, *J Biol Chem 263*, 7567, 1988 (*563*)
	Mouse, BALB/c, female, 6-8 week	Epithelial	Collagenase Type 3: 0.1%	DMEM	Ehmann, U., Peterson, W., and Misfeldt, D.: To Grow Mouse Mammary Epithelial Cells in Culture, *J Cell Biol 98*, 1026, 1984 (*926*)
	Mouse C3H/HeN, female	Epithelial	Deoxyribonuclease I: 0.0001%	Medium 199	Taketani, Y., and Oka, T.: EGF Stimulates Cell Proliferation and Inhibits Functional Differentiation of Mouse Mammalian Dipithelial Cells in Culture, *Endocrinology 113*, 871, 1983 (*380*)
	Mouse, BALB/c/Crgl Me, female, pregnant	Epithelial	Pepsin: 0.1% and 0.05%	HBSS with 0.2% EDTA, CMF	Riser, M., Huff, B., and Medina, D.: Pepsin Can Be Used To Subculture Viable Mammary Epithelial Cells, *In Vitro 19*, 730, 1983 (*532*)
	Mouse, female	Epithelial	Deoxyribonuclease I: 0.1%	DMEM	Jones, W., and Hallowes, R.: Isolation of the Epithelial Subcomponents of the Mouse Mammary Gland for Tissue-Level Culture Studies, *J Tiss Cul Meth 8 (1)*, 17, 1983 (*873*)
	Mouse NMuMG, female, 2 months	Epithelial	Collagenase Type 3: 0.1%	DMEM	Ehmann, U., and Misfeldt, D.: Mouse Mammary Cells in D-Valine Medium, *In Vitro 18*, 407, 1982 (*519*)

[CF=Calcium Free • CMF=Calcium Magnesium Free • BSS=Balanced Salt Solution]

Mammary (Con't)

Mammary (Con't)

Species		Cell(s)	Enzyme(s)	Medium	Reference
Mouse	Mouse BALB/cCrgl, virgin, female, 4-8 month	Epithelial Mammary gland	Collagenase Type 3: 0.1%	HBSS	White, M., Hu, A., Hamamoto, S. and Nandi: In vitro analysis of proliferating epithelial cell populations from the mouse mammary gland: fibroblast-free growth and serial passage, *In Vitro 14*, 271, 1978 (*498*)
	Mouse BALB/c, pregnant, 60-80 days	Mammary	Hyaluronidase: 0.1%	CMF medium	Ceriani, R., Peterson, J., and Abraham, S.: Removal of Cell Surface Material by Enzymes Used to Dissociate Mammary Gland Cells, *In Vitro 14*, 887, 1978 (*499*)
	Mouse, BALB/cCrgl, female also guinea-pig	Swiss 3T3	Collagenase Type 3: 0.2%	PBS CMF	Asch, B., and Medina, D.: Concanavalin A-Induced Agglutinability of Normal, Preneoplastic, and Neoplastic Mouse Mammary Cells, *J Natl Cancer Inst 61 (6)*, 1423, 1978 (*1012*)
	Mouse, lactating	Parenchymal	Collagenase Type 1: 0.3%	Kreb's Ringer bicarbonate buffer	Kerkof, P., and Abraham, S.: Preparation of Adipose Cell-Free Suspensions of Mammary Gland Parenchymal Cells from Lactating Mice, *Methods Enzymol 69*, 693, 1976 (*696*)
	Mouse, female, early pregnancy <10 days	Epithelial	Collagenase: 0.1%	Eagle's MEM	Moore, D., and Lasfargues, E.: Method for the Continuous Cultivation of Mammary Epithelium, *In Vitro 7*, 21, 1971 (*537*)
	Mouse, CBA, virgin, 9/10 wk	Mammary	Hyaluronidase: 0.1%	BSS, CMF	Prop, F., and Wiepjes, G.: Improved Method for Preparation of Single-cell Suspensions from Mammary Glands of Adult Virgin Mouse, *Exp Cell Res 61*, 451, 1970 (*400*)
	Mouse, lactating, 14-18 day	Parenchymal	Collagenase: 0.33%	Kreb's buffer	Pitelka, D., Kerkof, P., Gagne, H., Smith, S., and Abraham, S.: Characteristics of Cells Dissociated from Mouse Mammary Glands. I. Method of Separation and Morphology of Parenchymal Cells from Lactating Glands, *Exp Cell Res 57*, 43, 1969 (*398*)
	Mouse, lactating, 14 day	Mammary	Trypsin NF 1:250: 0.25%	HBSS	Kopelovich, L., Abraham, S., McGrath, H., DeOme, K., Chaikoff, I.: Metabolic Characteristics of a Naturally Ocurring Preneoplastic Tissue. I. Glycolytic Enzyme Activators of Hyperplastic Alveolar Nodule Outgrowths and Adenocarcinomas of Mouse Mammary Gland, *Cancer Res 26*, 1534, 1966 (*352*)
	Mouse, pregnant, 14-17 day	Mammary	Collagenase: 0.05% - 0.1%	HBSS	Daniel, C., and DeOme, K.: Growth of Mouse Mammary Glands *In Vivo* After Monolayer Culture, *Science 149*, 634, 1965 (*663*)
	Mouse, adult, 1-10 days pregnant	Epithelial Mammary	Collagenase: 0.02%	Simm's	Lasfargues, E.: Cultivation and Behavior *In Vitro* of the Normal Mammary Epithelium of the Adult Mouse. II. Observations on the Secretory Activity, *Exp Cell Res 13*, 553, 1957 (*390*)
Rat	Rat, female, 50 day	Mammary epithelial	Collagenase Type 3: 0.15%	DMEM/F12	Maffini M., Soto A., Calabro J., Ucci A., and Sonnenschein C.: The Stroma as a Crucial Target in Rat Mammary Gland Carcinogenesis, *J Cell Sci 117*, 1495, 2004 (*10001*)
	Rat, Lewis, female, 90 days	Epithelial Mammary	Collagenase: 0.05%	Medium 199	Lin, T., Hom, Y.K., Richards, J. and Nandi, S.: Effects of Antioxidants and Reduced Oxygen Tension on Rat Mammary Epithelial Cells in Culture, *In Vitro Cell Dev Biol 27A*, 191, 1991 (*458*)
	Rat, SD, female, 55 day	Epithelial	Neutral Protease: 3 u/ml	Medium 199	Ehmann, U., Osborn, R., Guzman, R., and Fajardo, L.: Cultured Proliferating Rat Mammary Epithelial Cells, *In Vitro Cell Dev Biol 27*, 749, 1991 (*477*)
	Rat, 50-60 days	Epithelial	Neutral Protease: 0.2%	EBSS	Hahm, H.A., Ip, M.M.: Primary Culture of Normal Rat Mammary Epithelial Cells Within a Basement Matrix. 1. Regulation of Proliferation by Hormones and Growth Factors, *In Vitro Cell Dev Biol 26*, 791, 1990 (*451*)
	Rat, Wistar, 13-18 day postpartum	Acini	Collagenase: 0.05%	HBSS	Katz, J., Wals, P. and Van de Velde, R: Lipogenesis by Acini from Mammary Gland of Lactating Rats, *J Biol Chem 249*, 7348, 1974 (*551*)
	Rat, SD, 15-20 day postpartum	Mammary	Collagenase: 0.2%	Kreb's Ringer bicarbonate buffer	Martin, R., and Baldwin, R.: Effects of Insulin on Isolated Rat Mammary Cell Metabolism: Glucose Utilization and Metabolite Patterns, *Endocrinology 89*, 1263, 1971 (*384*)

Miscellaneous

Miscellaneous

Species		Cell(s)	Enzyme(s)	Medium	Reference
Human	Human	Synoviocytes	Collagenase: 0.2%	DMEM/F12	Chen V, Croft D, Purkis P, Kramer IM: Co-culture of synovial fibroblasts and differentiated U937 cells is sufficient for high interleukin-6 but not interleukin-1beta or tumour necrosis factor-alpha release., *Br J Rheumatol Vol. 37*, 148-56, 1998 (*10360*)
	Human	Carotid artery plaque macrophage	Collagenase Type 4: 450 u/ml Deoxyribonuclease I: 500 u/ml Soybean Trypsin Inhibitor: 0.1%	HBSS	Patino Willmar D, Kang Ju-Gyeong, Matoba Satoaki, Mian Omar Y, Gochuico Bernadette R, Hwang Paul M: Atherosclerotic plaque macrophage transcriptional regulators are expressed in blood and modulated by tristetraprolin, *Circ Res 98*, 1282-9, 2006 (*10336*)
	Human	Periapical granuloma	CLSPA: 0.25%	RPMI-1640	Stern MH, Dreizen S, Mackler BF, Levy BM: Isolation and characterization of inflammatory cells from the human periapical granuloma, *J Dent Res 61*, 1408-12, 1982 (*10292*)
Insect	Insect, *Lepidoptera*	Lepitopteran	Collagenase Type 3: 0.35% Hyaluronidase: 0.01%	Dulbecco PBS	Goodwin, R and McCawley, P: Initiating Attached Cell Lines From the Lepidoptera (Insecta), *Meth Cell Sci 3*, 567, 1977 (*10675*)
Mouse	Mouse	Spleen, bone marrow endothelial	Collagenase Type 4: 0.3-1.0% Deoxyribonuclease I: 20 u/ml	PBS	Shi, C., Jia, T., Mendez-Ferrer, S., Hohl, T., Serbina, N., Lipuma, L., Leiner, I., Li, M., Frenette, P. and Pamer, E.: Bone Marrow Mesenchymal Stem and Progenitor Cells Induce Monocyte Emigration in Response to Circulating Toll-Like Receptor Ligands., *Immunity 34*, 590, 2011 (*10641*)
	Mouse, 25-30 g	Tracheal inflammatory cells	Collagenase Type 4: 0.1% Deoxyribonuclease I: 50 u/ml Soybean Trypsin Inhibitor: 0.1%	RPMI 1640	Minamoto Kanji, Pinsky DavidJ: Recipient iNOS but not eNOS deficiency reduces luminal narrowing in tracheal allografts, *J Exp Med 196*, 1321-33, 2002 (*10299*)
	Mouse, 6-8 week	Bone marrow mesenchymal stem	Collagenase Type 1: 0.25%	RPMI 1640	Xu, S., De Becker, A., Van Camp, B., Vanderkerken, K. and Van Riet, I.: An Improved Harvest and In Vitro Expansion Protocol for Murine Bone Marrow-Derived Mesenchymal Stem Cells., *J Biomed Biotechnol Vol. 2010*, 105940, 2010 (*10617*)
Rabbit	Rabbit, New Zealand, 8-10 week	Tenocytes and tendon stem cells	Collagenase Type 1: 0.3% Neutral Protease: 0.4%	DMEM	Zhang, J. and Wang, J.: Characterization of Differential Properties of Rabbit Tendon Stem Cells and Tenocytes., *BMC Musculoskelet Disord 11*, 10, 2010 (*10639*)

[CF=Calcium Free • CMF=Calcium Magnesium Free • BSS=Balanced Salt Solution]

Species		Cell(s)	Enzyme(s)	Medium	Reference
Rat	Rat, Wistar, 7-9 week	Gingival mitochondria	Collagenase Type 1: 0.115-0.130 %	HBSS	Kaneko, N., Rikimaru, T., Fujimura, T., Mori, S., Hidaka, S. and Kaya, H.: Preparation of Rat Gingival Mitochondria with an Improved Isolation Method., *Int J Dent Vol. 2010*, 275103, 2010 (*10603*)
	Rat, 8-10 week	Synovial cells	Collagenase Type 1: 250 u/ml	RPMI 1640	Moghaddami, M., Cleland, L. and Mayrhofer, G.: MHC II+ CD45+ Cells from Synovium-Rich Tissues of Normal Rats: Phenotype, Comparison with Macrophage and Dendritic Cell Lineages and Differentiation into Mature Dendritic Cells In Vitro., *Int Immunol 17*, 1103, 2005 (*10581*)

Muscle

Miscellaneous (Con't)

Muscle

Species		Cell(s)	Enzyme(s)	Medium	Reference
Bovine	Bovine	Heart, adrenal chromaffin paraneurons	Trypsin: 0.06% Deoxyribonuclease: 2 µ g/ml Collagenase: 0.05% Deoxyribonuclease: 13.25 µ g/ml	CMF 25mM HEPES buffered Locke's solution	Trifaro, J.-M., Tang, R., and Novas, M.L., Monolayer Co-Culture of Rat Heart Cells and Bovine Adrenal Chromaffin Paraneurons, *In Vitro Cell. Dev. Biol. 26*, 335-347, 1990
	Bovine	Smooth muscle, fibroblasts	Trypsin: 0.055%	DMEM	Davies, P. and Kerr, C.: Modification of LDL Metabolism by Growth Factors in Cultured Vascular Cells and Human Skin Fibroblasts, *Biochim Biophys Acta 712*, 26, 1982 (*322*)
Canine	Dog	Smooth muscle	Elastase: 50 u/ml	PSS	Subramanian, M., Madden, J., and Harder, D.: A Method for the Isolation of Cells from Arteries of Various Sizes, *J Tiss Cul Meth 13*, 13, 1991 (*1240*)
	Dog, beagle, adult	Smooth muscle Vascular	Elastase: 34 u/ml	Tyrode's solution w/ calcium	Wilde, D., and Lee, K.: Outward Potassium Currents in Freshly Isolated Smooth Muscle Cell of Dog Coronary Arteries, *Circ Res 65*, 1718, 1989 (*368*)
Chicken	Chicken, 1-2 day	Gizzard and aorta smooth muscle	Collagenase Type 1: 0.15%	HBSS	Dirksen W., Vladic F., and Fisher S.: A Myosin Phosphatase Targeting Subunit Isoform Transition Defines a Smooth Muscle Developmental Phenotypic Switch, *Am J Physiol/Cell 278(3)*, C589, 2000 (*9837*)
	Chick	Smooth muscle	Trypsin: 0.05% - 0.1%	HBSS	Chamley-Campbell, J., Campbell, G., and Ross, R.: The Smooth Muscle in Cell Culture, *Physiol Res 59*, 1, 1979 (*648*)
	Chick, white leghorn, 12 day	Muscle	Trypsin: 0.25%	Puck's saline A	Bullaro, J., and Brookman, D.: Comparison of Skeletal Muscle Monolayer Cultures Initiated With Cells Dissociated by the Vortex and Trypsin Methods, *In Vitro 12*, 564, 1976 (*497*)
	Chick, white leghorn, embryos, 11 day	Muscle	Trypsin: 0.05%	Saline G	Tepperman, K., Morris, G., Essien, F., and Heywood, S.M.: A Mechanical Dissociation Method For Preparation of Muscle Cell Cultures, *J Cell Physiol 86*, 561, 1975 (*597*)
	Chick embryo	Thyroid Muscle Heart	Collagenase: 0.25%	Tyrode's saline, potassium free	Hilfer, S., and Brown, J.: Collagenase. Its Effectiveness as a Dispersing Agent for Embryonic Chick Thyroid and Heart, *Exp Cell Res 65*, 246, 1971 (*401*)
	Chick, embryonic	Muscle	Trypsin: 0.1%	CMF HBSS	Hilfer, S.: Collagenase Treatment of Chick Heart and Thyroid,*Tissue Cult Methods & Applications*,Kruse, P., and Patterson, M., 246, 1971 (*1283*)
	Chick embryonic	Various tissues (heart, liver, skeletal, cardiac)	Trypsin: various grades	CMF Tyrode's solution	Rinaldini, L.: An Improved Method for the Isolation and Quantitative Cultivation of Embryonic Cells, *Exp Cell Res 16*, 477, 1959 (*394*)
Feline	Cat, adult mongrel, either sex, 2.5-4.0 kg	Cerebral arteries	Elastase: 50 u/ml	Puck's solution	Madden, J., Vadula, M., and Kurup, V.: Effects of Hypoxia and Other Vasoactive Agents on Pulmonary and Cerebral Artery Smooth Muscle Cells, *Am J Physiol 263*, L384, 1992 (*778*)
Fish	Dogfish, *Squalus acanthias* , 2-6 Kg (also guinea-pig, rabbit, frog)	Myocytes, heart and stomach	Protease XIV: 0.028%	Solution C (see reference)	Mitra, R. and Morad, M.: A Uniform Enzymatic Method for Dissociation of Myocytes from Hearts and Stomachs of Vertebrates, *Am J Physiol 249*, H1056, 1985 (*294*)
Frog	Frog, *Xenopus laevis*, embryos stage 17 & 19	Muscle	Collagenase: 0.10%	Steinberg's solution	Stollberg, J. and Fraser, S.: Acetylcholine Receptors and Con A-Binding Sites on *Xenopus* Muscle Cells, *J Cell Biol 107*, 1397, 1988 (*579*)
	Frog	Myocytes	Trypsin: 0.1%	CF Ringer	Shepherd, N. and Kavaler, F.: Direct Control of Contraction Force of Single Frog Atrial Cells by Extracellular Ions, *Am J Physiol 251*, C653, 1986 (*296*)
	Frog, *Rana pipiens* (50-100 g) (also guinea-pig, rabbit, dogfish, *Squalus acanthias*)	Myocytes, heart and stomach	Protease XIV: 0.028%	Solution C (see reference)	Mitra, R. and Morad, M.: A Uniform Enzymatic Method for Dissociation of Myocytes from Hearts and Stomachs of Vertebrates, *Am J Physiol 249*, H1056, 1985 (*294*)
	Frog, *Xenopus laevis*	Muscle	Trypsin: 0.5%	L15 medium (see reference)	Anderson, M.J., Cohen, M.W., and Zorychta, E.: Effects of Innervation on the Distribution of Acetylcholine Receptors on Cultured Muscle Cells, *J Physiol 268*, 731, 1977 (*722*)
Guinea-Pig	Guinea-pig	Bladder smooth muscle	Collagenase Type 2: 0.1-0.2%	Krebs-Ringer bicarbonate	Shieh CC, Feng J, Buckner SA, Brioni JD, Coghlan MJ, Sullivan JP, Gopalakrishnan M: Functional implication of spare ATP-sensitive K(+) channels in bladder smooth muscle cells, *J Pharmacol Exp Ther 296*, 669-75, 2001 (*10238*)
	Guinea-pig, 200-380 g	Capillaries Myocytes	Collagenase Type 2: 0.15%	CF solution	Schnitzler, M., Derst, C., Daut, J., and Preisig-Muller, R.: ATP-Sensitive Potassium Channels in Capillaries Isolated From Guinea-Pig Heart, *J Physiol 525 (2)*, 307, 2000 (*744*)
	Guinea pig, 2-4 wk old, male, female	Smooth muscle Gallbladder	Papain: 0.1%	Krebs solution	Firth, T., Mawe, G., and Nelson, M.: Pharmacology and Modulation of K_{ATP} Channels by Protein Kinase C and Phosphatates in Gallbladder Smooth Muscle, *Am J Physiol Cell Physiol 278*, C1031, 2000 (*1131*)
	Guinea-pig, adult, 250-350 g	Smooth muscle Gallbladder	Papain: 0.1%	NaCl, sodium glutamate, MgCl, KCl, glucose, Kreb's, and HEPES	Jennings, L., Xu, Q., Firth, T., Nelson, M., and Mawe, G.: Cholesterol Inhibits Spontaneous Action Potentials and Calcium Currents in Guinea Pig Gallbladder Smooth Muscle, *Am J Physiol 277*, G1017, 1999 (*1114*)
	Guinea-pig, Dunkin-Hartley, female	Myocytes	Protease:	DMEM	Ryder, K., Bryant, S., and Hart, G.: Membrane Current Changes in Left Ventricular Myocytes Isolated From Guinea-Pigs After Abdominal Aortic Coarctation, *Cardiovasc Res 27*, 1278, 1993 (*970*)
	Guinea-pig (also rat, rabbit)	Smooth muscle	Trypsin: 0.1%	Potassium buffer solution	Hu, S., and Kim, H.: Activation of K+ Channel in Vascular Smooth Muscle by Cytochrome P-450 Metabolites of Arachidonic Acid, *FASEB J 6*, A383, 1992 (*409*)

[CF=Calcium Free • CMF=Calcium Magnesium Free • BSS=Balanced Salt Solution]

Muscle (Con't)

Species		Cell(s)	Enzyme(s)	Medium	Reference
Guinea-Pig	Guinea-pig, 200-400 g (also rabbit, frog, dogfish)	Myocytes, heart and stomach	Protease XIV: 0.028%	Solution C (see reference)	Mitra, R. and Morad, M.: A Uniform Enzymatic Method for Dissociation of Myocytes from Hearts and Stomachs of Vertebrates, *Am J Physiol 249*, H1056, 1985 (*294*)
	Guinea-pig, prepubertal	Smooth muscle Aortic	Trypsin: 0.05%	Dulbecco-Vogt modification of Eagle's	Ross, R.: The Smooth Muscle Cell . II. Growth of Smooth Muscle in Culture and Formation of Elastic Fibers, *J Cell Biol 50*, 172, 1971 (*584*)
Hamster	Hamster, male, 60-70 day	Satellite	Trypsin: 0.25%	DMEM	Nakamura, T., Iwata, Y., Sampaolesi, M., Hanada, H., Saito, N., Artman, M., Coetzee, W., and Shigekawa, M.: Stretch-Activated Cation Channels in Skeletal Muscle Myotubes From Sarcoglycan-Deficient Hamsters, *Am J Physiol Cell Physiol 281*, C690, 2001 (*747*)
Human	Human, male	Myogenic	Collagenase Type 4: 0.1% Neutral Protease: 2.4 u/ml	HBSS	Stadler, G., Chen, J., Wagner, K., Robin, J., Shay, J., Emerson, C. and Wright, W.: Establishment of Clonal Myogenic Cell Lines from Severely Affected Dystrophic Muscles - CDK4 Maintains the Myogenic Population., *Skelet Muscle 1*, 12, 2011 (*10667*)
	Human	Muscle derived multiprogenitor cells	Collagenase Type 2: 0.05%	DMEM	Nesti, L., Jackson, W., Shanti, R., Koehler, S., Aragon, A., Bailey, J., Sracic, M., Freedman, B., Giuliani, J. and Tuan, R.: Differentiation Potential of Multipotent Progenitor Cells Derived from War-Traumatized Muscle Tissue., *J Bone Joint Surg Am Vol. 90*, 2390, 2008 (*10490*)
	Human	Endothelial and vascular smooth muscle	Collagenase Type 1: 0.2%	HBSS	Moss, S., Bates, M., Parrino, P. and Woods, TC.: Isolation of Endothelial Cells and Vascular Smooth Muscle Cells from Internal Mammary Artery Tissue., *Ochsner J 7*, 133, 2007 (*10636*)
	Human	Urinary tract smooth muscle	Collagenase Type 4: 100 u/ml	DMEM	Kimuli, M., Eardley, I. and Southgate, J.: In Vitro Assessment of Decellularized Porcine Dermis as a Matrix for Urinary Tract Reconstruction., *BJU Int Vol. 94*, 859, 2004 (*10570*)
	Human, female	Smooth muscle Myometrial	Deoxyribonuclease I: 0.015% and 0.007%	HBSS	Richardson, M., Taylor, D., Casey, M., MacDonald, P., and Stull, J.: Biochemical Markers of Contraction in Human Myometrial Smooth Muscle Cells in Culture, *In Vitro Cell Dev Biol 23*, 21, 1987 (*420*)
	Human, female	Smooth muscle Myometrial	Deoxyribonuclease I: 0.12%	HBSS	Casey, M., MacDonald, P., Mitchell, M., and Snyder, J.: Maintenance and Characterization of Human Myometrial Smooth Muscle Cells in Monolayer Culture, *In Vitro 20*, 396, 1984 (*533*)
	Human, fetal (also bovine)	Smooth muscle, fibroblasts	Trypsin: 0.055%	DMEM	Davies, P. and Kerr, C.: Modification of LDL Metabolism by Growth Factors in Cultured Vascular Cells and Human Skin Fibroblasts, *Biochim Biophys Acta 712*, 26, 1982 (*322*)
	Human	Smooth muscle	Trypsin: 0.25%	DMEM	Eskin, S., Sybers, H., Lester, J., Navarro, L., Gotto, A., and DeBakey, M.: Human Smooth Muscle Cells Cultured From Atherosclerotic Plaques and Uninvolved Vessel Wall, *In Vitro 17 (8)*, 713, 1981 (*864*)
	Human (also rat, guinea-pig, chick, monkey)	Smooth muscle	Trypsin: 0.05% - 0.1%	HBSS	Chamley-Campbell, J., Campbell, G., and Ross, R.: The Smooth Muscle in Cell Culture, *Physiol Res 59*, 1, 1979 (*648*)
Lizard	Lizard (*Anolis carolinensis*)	Myoblasts, tail	Collagenase: 0.2%	GM III (see reference)	Cox, P., and Simpson, Jr., S.: A Microphotometric Study of Myogenic Lizard Cells Grown in Vitro, *Dev Biol 23*, 433, 1970 (*369*)
Monkey	Monkey (*Macaca nemestrina*)	Smooth muscle	Trypsin: 0.05%	Dulbecco-Vogt	Chait, A., Ross, R., Albers, J., and Bierman, E.: Platelet-Derived Growth Factor Stimulates Activity of LDL Receptors, *Proc Natl Acad Sci U S A 77*, 4084, 1980 (*654*)
	Rhesus monkey, 1 year (also human, rabbits)	Smooth muscle, saphenous vein	Elastase: 0.05%	BSS	Chamley, J., Campbell, G., McConnell, J., and Groschel-Stewart, U.: Comparison of Vascular Smooth Muscle Cells from Adult Human, Monkey and Rabbit in Primary Culture and in Subculture, *Cell Tissue Res 177*, 503, 1977 (*354*)
Mouse	Mouse, 1-25 month	Myocytes, endothelial	Neutral Protease: 1.2 u/ml Collagenase Type 4: 0.2%	PBS	Ieronimakis Nicholas, Balasundaram Gayathri, Reyes Morayma: Direct isolation, culture and transplant of mouse skeletal muscle derived endothelial cells with angiogenic potential, *PLoS ONE 3*, e0001753, 2008 (*10313*)
	Mouse, 15-20 day	Intersitial cells of Cajal	Collagenase Type 2: 0.13%	M199	Li CX, Liu BH, Tong WD, Zhang LY, and Jiang YP: Dissociation, culture and morphologic changes of interstitial cells of Cajal in vitro, *World J Gastroenterol 11*, 2838, 2005 (*10007*)
Ovine	Sheep, adult and neonatal	Tracheal smooth muscle cells	Papain: 0.2% Deoxyribonuclease I: 0.1%	MOPS-PSS	Driska S., Laudadio R., Wolfson M., and Shaffer T.: A Method for Isolating Adult and Neonatal Airway Smooth Muscle Cells and Measuring Shortening Velocity, *J Appl Physiol 86(1)*, 427, 1999 (*9841*)
Porcine	Porcine	Smooth muscle, aorta	Collagenase: 0.3%	DMEM	Xiong, Yimin, Xu, Shangzhe, and Slakey, Linda L: Modulation of Response to Adenosine in Vascular Smooth Muscle Cells Cultured in Defined Medium, *In Vitro Cell Dev Biol 27*, 355, 1991 (*463*)
	Porcine	Smooth muscle Aorta	Trypsin: 0.05%	EDTA 0.02%	Breton, M., Berrou, E., Deudon, E. and Picard, J.: Changes in Proteoglycans of Cultured Pig Aortic Smooth Muscle Cells During Subculture, *In Vitro Cell Dev Biol 26*, 157, 1990 (*431*)
	Porcine	Smooth muscle Aortic medial tissue	Collagenase: 0.30%	DMEM	Fehr, T., Dickinson, E., Goldman, S. and Slakey, L.: Cyclic AMP Efflux is Regulated by Occupancy of The Adenosine Receptor in Pig Aortic Smooth Muscle Cells, *J Biol Chem 265*, 10974, 1990 (*566*)
Quail	Quail, embryo, 10 day	Myoblasts	Collagenase Type 2: 0.1%	Puck's solution	Konigsberg, I.: Skeletal Myoblasts in Culture, *Methods Enzymol LVIII*, 511, 1979 (*638*)
Rabbit	Rabbit, New Zealand White	Aortic smooth muscle	Collagenase Type 2: 300 u/ml Elastase: 5 u/ml	F10 Ham's	Croons Valerie, Martinet Wim, Herman Arnold G, Timmermans Jean-Pierre, De Meyer Guido R Y: Selective clearance of macrophages in atherosclerotic plaques by the protein synthesis inhibitor cycloheximide, *J Pharmacol Exp Ther 320*, 986-93, 2007 (*10348*)
	Rabbit (also rat, guinea-pig)	Smooth muscle	Trypsin: 0.1%	Potassium buffer solution	Hu, S., and Kim, H.: Activation of K+ Channel in Vascular Smooth Muscle by Cytochrome P-450 Metabolites of Arachidonic Acid, *FASEB J 6*, A383, 1992 (*409*)
	Rabbit, adult, 1-2 kg	Smooth muscle	Elastase: 0.17 - 0.25%	Saline	Benham, C., Bolton, T., Byrne, N., and Large, W.: Action of Extremely Applied Adenosine Triphosphate O Single Smooth Muscle Cells Dispersed From Rabbit Ear Artery, *J Physiol 387*, 473, 1987 (*863*)
	Rabbit, New Zealand white, 1500 g	Smooth muscle, aortic	Trypsin: 0.038%	MEM	Knodle, S., Anderson, S., and Papaioannou, S.: Large Scale Preparation of Rabbit Aortic Smooth Muscle Cells For Use in Calcium Uptake Studies, *In Vitro Cell Dev Biol 22*, 23, 1986 (*416*)
	Rabbit, adult, 1-2 kg	Smooth muscle, ear artery	Trypsin: 0.1%	CF solution (see reference)	Benham, C.D., Bolton, T.B.: Spontaneous Transient Outward Currents in Single Visceral and Vascular Smooth Muscle Cells of the Rabbit, *J Physiol 381*, 385, 1986 (*720*)

[CF=Calcium Free • CMF=Calcium Magnesium Free • BSS=Balanced Salt Solution]

Muscle (Con't)

Species		Cell(s)	Enzyme(s)	Medium	Reference
Rabbit	Rabbit, 0.5-1 kg (also guinea-pig, frog, dogfish)	Myocytes, heart and stomach	Protease XIV: 0.028%	Solution C (see reference)	Mitra, R. and Morad, M.: A Uniform Enzymatic Method for Dissociation of Myocytes from Hearts and Stomachs of Vertebrates, *Am J Physiol 249*, H1056, 1985 (*294*)
	Rabbit, white New Zealand, adult, male, 2 kg	Smooth muscle, aorta	Trypsin: 0.1%	Krebs Ringer HEPES solution	Ives, H., Schultz, G., Galardy, R., and Jamieson, J.: Preparation of Functional Smooth Muscle Cells from the Rabbit Aorta, *J Exp Med 148*, 1400, 1978 (*603*)
	Rabbits, New Zealand white albino, 5-6 months (also human, Rhesus monkey, rabbit)	Smooth muscle, saphenous vein	Elastase: 0.05%	BSS	Chamley, J., Campbell, G., McConnell, J., and Groschel-Stewart, U.: Comparison of Vascular Smooth Muscle Cells from Adult Human, Monkey and Rabbit in Primary Culture and in Subculture, *Cell Tissue Res 177*, 503, 1977 (*354*)
	Rabbit, chinchilla, 5-8 month, virgin, female, 2-3 kg	Smooth muscle, aorta	Hyaluronidase: 800 u/ml	HBSS	Peters, T., Muller, M., and deDuve, C.: Lysosomes of the Arterial Wall. I. Isolation and Subcellular Fractionation of Cells from Normal Rabbit Aorta, *J Exp Med 136*, 1117, 1972 (*601*)
	Rabbit, New England, albino	Thoracic aorta	Elastase: 0.008%	Kreb's Ringer	Day, A., Phil, D., and Newman, H.: Synthesis of Phospholipid by Foam Cells Isolated from Rabbit Atherosclerotic Lesions, *Circ Res XIX*, 122, 1966 (*777*)
Rat	Rat, fetal 17-18 day	Myotubes	Trypsin: 0.05%	(see reference)	Das, M., Rumsey, J., Bhargava, N., Stancescu, M. and Hickman, J.: Skeletal Muscle Tissue Engineering: A Maturation Model Promoting Long-Term Survival of Myotubes, Structural Development of the Excitation-Contraction Coupling Apparatus and Neonatal Myosin Heavy Chain Expression., *Biomaterials 30*, 5392, 2009 (*10654*)
	Rat, Lewis, neonatal	Myoblasts	Collagenase Type 2: 1.0% Neutral Protease: 2.4 u/ml	Ham's F-10	Kim, J., Hadlock, T., Cheney, M., Varvares, M. and Marler, J.: Muscle Tissue Engineering for Partial Glossectomy Defects., *Arch Facial Plast Surg 5*, 403, 2003 (*10637*)
	Rat, SD, adult and neonatal w/in 1 day of birth	Myooids	Neutral Protease: 4 u/ml	Ham's F-12	Dennis, R., Kosnik II, P., Gilbert, M., and Faulkner, J.: Excitability and Contractility of Skeletal Muscle Engineered from Primary Cultures and Cell Lines, *Am J Physiol Cell Physiol 280*, C288, 2001 (*1111*)
	Rat, SD, 3 month	Smooth muscle cells	Collagenase Type 2: 0.2% Elastase: 0.04% Soybean Trypsin Inhibitor: 0.1%	M-199	Su E., Stevenson S., Rollence M., Marshall-Neff J., and Liau G.: A Genetically Modified Adenoviral Vector Exhibits Enhanced Gene Transfer of Human Smooth Muscle Cells, *J Vasc Res 38(5)*, 471, 2001 (*9860*)
	Rat, SD, 250 g	Arterial smooth muscle	Papain: 0.03% Collagenase: 0.1%	(see reference)	Jaggar JH: Intravascular pressure regulates local and global Ca(2+) signaling in cerebral artery smooth muscle cells, *Am J Physiol Cell Physiol 281*, C439-48, 2001 (*10325*)
	Rat, Wistar, adult, male	Smooth & skeletal muscle Cardiac myocytes	Protease: 0.01%	PSS	Wellman, G., Barrett-Jolley, R., Koppel, H. Everitt, D., and Quayle, J.: Inhibition of Vascular K_{ATP} Channels by U-37883A: A Comparison with Cardiac and Skeletal Muscle, *Br J Pharmacol 128*, 909, 1999 (*1065*)
	Rat, SD, male, 250-350 g	Vascular smooth muscle	Collagenase Type 2: 0.1% Elastase: 0.0125%	DMEM	Hrometz, S., Edelmann, S., McCune, D., Olges, J., Hadley, R., Perez ,D., and Piascik, M.: Expression of Multiple Alpha1-Adrenoceptors on Vascular Smooth Muscle: Correlation with the Regulation of Contraction, *J Pharmacol Exp Ther 290(1)*, 452, 1999 (*9867*)
	Rat, SD, male, 150-175 g	Smooth muscle, endothelial	Trypsin: 0.04%	MEM	Redmond, E., Cahill, P., and Sitzmann, J.: Perfused Transcapillary Smooth Muscle and Endothelial Cell Co-Culture-A Novel *In Vitro* Model, *In Vitro Cell Dev Biol Anim 31*, 601, 1995 (*1234*)
	Rat, Wistar Kyoto, 10-15 weeks	Smooth muscle, mesenteric artery	Trypsin: 0.05%	MEM	McGuire, P., Walker-Caprioglio, H., Little, S., and McGuffee, L.: Isolation and Culture of Rat Superior Mesenteric Artery Smooth Muscle Cells, *In Vitro Cell Dev Biol 29*, 135, 1993 (*491*)
	Rat (also rabbit, guinea-pig)	Smooth muscle	Trypsin: 0.1%	Potassium buffer solution	Hu, S., and Kim, H.: Activation of K+ Channel in Vascular Smooth Muscle by Cytochrome P-450 Metabolites of Arachidonic Acid, *FASEB J 6*, A383, 1992 (*409*)
	Rat, SD, 19 days	Smooth muscle, myometrial	Trypsin: 150 µg/ml	HBSS or PSS, CMF	Loch-Caruso, R., Pahl, M., and Juberg, D.: Rat Myometrial Smooth Muscle Cells Show High Levels of Gap Junctional Communication Under a Variety of Culture Conditions, *In Vitro Cell Dev Biol 28*, 97, 1992 (*489*)
	Rat, SHRs and WKY, male, 10 - 14 weeks	Smooth muscle, tail arteries	Papain: 0.1%	HEPES buffer (see reference)	Bolzon, B. and Cheung, D.: Isolation and Characterization of Single Vascular Smooth Muscle Cells From Spontaneously Hypertensive Rats, *Hypertension 14*, 137, 1989 (*694*)
	Rat, 1-3 day	Smooth muscle, aortic	Elastase: 0.0125%	DMEM	Barone, L., Wolfe, L., Faris, B., and Franzblau, C.: Elastin mRNA Levels and Insoluble Elastin Accumulation in Neonatal Rat, *Biochem 27*, 3175, 1988 (*313*)
	Rat	Muscle, mesenteric arteries	Trypsin: 0.05%	HEPES KG solution (see reference)	Bean, B., Sturek, M., Puga, A., and Hermsmeyer, K.: Calcium Channels in Muscle Cells Isolated From Rat Mesenteric Arteries: Modulation by Dihydropyridine Drugs, *Circ Res 59*, 229, 1986 (*364*)
	Rat, SHR, WKY. either sex, 12-19 day, 3 month, and retired breeders	Endothelial, aortic	Elastase: 0.05%	Waymouth's culture medium	Gordon, D., Mohai, L., and Schwartz, S.: Induction of Polyploidy in Cultures of Neonatal Rat Aortic Smooth Muscle Cells, *Circ Res 59*, 633, 1986 (*866*)
	Rat, male, 150-250 g	Endothelial, aortic	Trypsin:	RPMI 1640	Cole, O., Fan, T., and Lewis, G.: Isolation, Characterization, Growth and Culture of Endothelial Cells From the Rat Aorta, *Cell Biol Int Rep 10 (6)*, 399, 1986 (*884*)
	Rat, Wistar, female, 10 weeks	Myocytes	Collagenase: 0.1%	HBSS	Boulanger-Saunier, C., Kattenburg, D., and Stoclet, J.: Cyclic AMP-dependent Phosphorylation of a 16kDa Protein in a Plasma Membrane-enriched Fraction of Rat Aortic Myocytes, *FEBS Lett 193*, 283, 1985 (*411*)
	Rat, SD, male, 200 - 250 g	Smooth muscle, thoracic aorta	Trypsin: 0.0375%	Eagle's MEM with calcium	Brock, T., Alexander, R., Ekstein, L., Atkinson, W., and Gimbrone, M.: Angiotensin Increases Cytosolic Free Calcium in Cultured Vascular Smooth Muscle Cells, *Hypertension 7*, 105, 1985 (*693*)
	Rat, SD, 225-250g	Mesenteric artery smooth muscle cells	Elastase: .0125% Soybean Trypsin Inhibitor: 0.025% Collagenase Type 1: 0.1%	HBSS	Gunther S, Alexander RW, Atkinson WJ, and Gimbrone MA Jr.: Functional angiotensin II receptors in cultured vascular smooth muscle cells, *J Cell Biol 92*, 289, 1982 (*10058*)
	Rat, 3-4 day	Myocardial	Trypsin NF 1:250: 0.125%	HBSS CMF	Kasten, F.: Rat Myocardial Cells *In Vitro*: Mitosis and Differentiated Properties, *In Vitro 8*, 128, 1972 (*539*)
	Rat, 200 g	Muscle	Trypsin: 0.05%	Kreb's Henseleit bicarbonate buffer	Kono, T.: Roles of Collagenases and Other Proteolytic Enzymes in the Dispersal of Animal Tissues, *Biochim Biophys Acta 178*, 397, 1969 (*317*)
	Rat, Wistar, 3-10 day	Heart	Trypsin NF 1:250: 250: 0.1%	Saline A (see reference)	Harary, I., and Farley, B.: *In Vitro* Studies on Single Beating Rat Heart Cells, *Exp Cell Res 29*, 451, 1963 (*395*)

[CF=Calcium Free • CMF=Calcium Magnesium Free • BSS=Balanced Salt Solution]

Species		Cell(s)	Enzyme(s)	Medium	Reference
Bovine	Bovine (also rat)	Heart Adrenal chromaffin Paraneurons	Trypsin: 0.06%	25mM HEPES buffered Locke's solution, CMF	Trifaro, J., Tang, R., and Novas, M.: Monolayer Co-Culture of Rat Heart Cells and Bovine Adrenal Chromaffin Paraneurons, *In Vitro Cell Dev Biol 26*, 335, 1990 (*438*)
	Bovine	Microvascular endothelial	Collagenase/Dispase: 0.1%	MEM	Bowman, P., Betz, A., and Goldstein, G.: Primary Culture of Microvascular Endothelial Cells From Bovine Retina, *In Vitro 18 (7)*, 626, 1982 (*945*)
	Calf (also lamb)	Oligodendroglia Neural	Trypsin: 0.1%	(see reference)	Poduslo, S., Miller, K., and McKhann, G.: Metabolic Properties of Maintained Oligodendroglia Purified from Brain, *J Biol Chem 253*, 1592, 1978 (*552*)
Chicken	Chick, embryo, 10-14 day	Flat, retina	Trypsin: 0.1%	Tyrode's solution, CMF	Moyer, M., Bullrich, F., and Sheffield, J.: Emergence of Flat Cells From Glia in Stationary Cultures of Embryonic Chick Neural Retina, *In Vitro Cell Dev Biol 26*, 1073, 1990 (*427*)
	Chick, White Leghorn, embryo (also rat, SD, embryo)	Spinal cord	Trypsin: 0.05%	Phosphate buffer (see reference)	Schnaar, R., and Schaffner, A.: Separation of cell types from embryonic chicken and rat spinal cord: characterization of motoneuron-enriched fractions, *J Neurosci 1*, 204, 1981 (*610*)
	Chick	Dorsal root ganglion neurons Spinal cord	Trypsin: 0.1%	Puck's saline, CMF	Choi, D., and Fiscbach, G.: GABA Conductance of Chick Spinal Cord and Dorsal Root Ganglion Neurons in Cell Culture, *J Neurophysiol 45*, 605, 1981 (*717*)
	Chick, White Leghorn embryo, 8 day	Neurons, ganglia	Trypsin: 0.25%	HBSS, CMF	Bottenstein, J., Skaper, S., Varon, S., and Sato, G.: Selective Survival of Neurons from Chick Embryo Sensory Ganglionic Dissociates Utilizing Serum-Free Supplemented Medium, *Exp Cell Res 125*, 183, 1980 (*388*)
	Chick embryos	Ganglion chains, sympathetic ganglia	Trypsin: 0.25%	Krebs Phosphosaline	McCarthy, K., and Partlow, L.: Preparation of Pure Neuronal and Non-Neuronal Cultures From Embryonic Chick Sympathetic Ganglia: A New Method Based on Both Differential Cell Adhesiveness and the Formation of Homotypic Neuronal Aggregates, *Brain Res 114*, 391, 1976 (*345*)
Fish	Black ghose knige fish, adult (*Apteronotus albifrons*)	Neurons, spinal cord	Trypsin: 0.4%	PBS, CMF	Anderson, M.J.: Differences in Growth of Neurons from Normal and Regenerated Teleost Spinal Cord in vitro, *In Vitro Cell Dev Biol 29A*, 145, 1993 (*492*)
Frog	*Xenopus*, embryonic	Neuron	Collagenase Type 1: 0.1%	Steinberg's solution	Takahashi, T., Nakajima, Y., Hirosawa, K., Nakajima, S., and Onodera, K.: Structure and Physiology of Developing Neuromuscular Synapses in Culture, *J Neurosci 7*, 473, 1987 (*619*)
Guinea-Pig	Guinea-pig, newborn	Neuron, enteric	Trypsin: 0.125%	Medium 199	Jessen, K, McConnell, J., Purves, R., Burnstock, G., and Chamley-Campbell, J.: Tissue Culture of Mammalian Enteric Neurons, *Brain Res 152*, 573, 1978 (*347*)
Hamster	Hamster, male	Vomeronasal organ neurons	Collagenase Type 1: 0.02% Trypsin: 0.02%	PBS	Liman ER: Regulation by voltage and adenine nucleotides of a Ca2+-activated cation channel from hamster vomeronasal sensory neurons, *J Physiol 548*, 777, 2003 (*10044*)
Human	Human	Neural, various	Papain: 12 u/ml Trypsin: see reference Collagenase/Dispase: see reference	(see reference)	Panchision David M, Chen Hui-Ling, Pistollato Francesca, Papini Daniela, Ni Hsiao-Tzu, Hawley Teresa S: Optimized flow cytometric analysis of central nervous system tissue reveals novel functional relationships among cells expressing CD133, CD15, and CD24, *Stem Cells 25*, 1560-70, 2007 (*10297*)
	Human, adult	Ventricular epithelial	Papain: 11.4 u/ml Deoxyribonuclease I: 10 u/ml	DMEM/F12	Roy NS, Benraiss A, Wang S, Fraser RA, Goodman R, Couldwell WT, Nedergaard M, Kawaguchi A, Okano H, Goldman SA: Promoter-targeted selection and isolation of neural progenitor cells from the adult human ventricular zone, *J Neurosci Res 59*, 321, 2000 (*10038*)
Insect	Drosophila	Dendrites	Collagenase: 0.05% Neutral Protease: 0.2%	HBSS	Sanchez-Soriano, N., Bottenberg, W., Fiala, A., Haessler, U., Kerassoviti, A., Knust, E., Lohr, R. and Prokop, A.: Are Dendrites in Drosophila Homologous to Vertebrate Dendrites?, *Dev Biol 288*, 126, 2005 (*10367*)
Mouse	Mouse, embryonic	Dopaminergic neurons	Trypsin: 0.1% Deoxyribonuclease I: 0.02%	DMEM	Radad Khaled, Gille Gabriele, Rausch Wolf-Dieter: Dopaminergic neurons are preferentially sensitive to long-term rotenone toxicity in primary cell culture, *Toxicol In Vitro 22*, 68-74, 2008 (*10347*)
	Mouse, 6 mo	Neurons, neurospheres	Papain: 0.2%	Hibernate	Brewer Gregory J, Torricelli John R: Isolation and culture of adult neurons and neurospheres, *Nat Protoc 2*, 1490-8, 2007 (*10095*)
	Mouse, C57BL/6, 1 year	Neurons	Papain: 0.2%	DMEM	Eide, L, and McMurray, C: Culture of Adult Mouse Neurons, *Biotechniques 38(1)*, 99-104, 2005 (*9787*)
	Mouse, adult	Brain and spinal cord cells	Trypsin: 0.25%	PBS	Gonzalez John M, Bergmann Cornelia C, Fuss Babette, Hinton David R, Kangas Cindy, Macklin Wendy B, Stohlman Stephen A: Expression of a dominant negative IFN-gammareceptor on mouse oligodendrocytes, *Glia 51*, 22-34, 2005 (*10111*)
	Mouse, CD-1, neonate	Neurons, dorsal root ganglion	Trypsin: 0.25%	HBSS	Quinn, S. and De Boni, U.: Enhanced Neuronal Regeneration by Retinoic Acid of Murine Dorsal Root Ganglia and of Fetal Murine and Human Spinal Cord in vitro, *In Vitro Cell Dev Biol 27*, 55, 1991 (*468*)
	Mouse, fetal	Precursor	Trypsin: 0.5%	PBS	Kitani, H., Shiurba, R., Sakakura, T., Tomooka, Y.: Isolation and Characterization Of Mouse Neural Precursor Cells in Primary Culture, *In Vitro Cell Dev Biol 27*, 615, 1991 (*470*)
	Mouse (SWR or CF1), 1-3 months	Papillae, taste receptor	Pronase E: 0.15%	Carbonate-Phosphate buffer (see reference)	Spielman, A., Mody, I., Brand, J., Whitney, G., MacDonald, J., and Salter, M.: A Method for Isolating and Patch-Clamping Single Mammalian Taste Receptor Cells, *Brain Res 503*, 326, 1989 (*350*)
	Mouse, neonatal (also chick)	PNS test neurons	Trypsin: 0.08%	Eagle's Basal Medium (see reference)	Varon, S., Skaper, S., Barbin, G., Selak, I., and Manthorpe, M.: Low Molecular Weight Agents Support Survival of Cultured Neurons From the Central Nervous System, *J Neurosci 4 (3)*, 654, 1984 (*1000*)
	Mouse (BALB/c), adult	Neurons, spinal cord	Collagenase Type 3: 0.25%	Hank's BSS, CMF	Eagleson, K. and Bennett, M.: Survival of Purified Motor Neurons *In Vitro*: Effects of Skeletal Muscle-Conditioned Medium, *Neurosci Lett 38*, 187, 1983 (*645*)
	Mouse, 0-30 day	Neural	Trypsin NF 1:250: 50 0.25%	BSS	Shrier, B., Wilson, S., and Nirenberg, M.: Cultured Cell Systems and Methods for Neurobiology, *Vol. 32*, 765, 1974 (*637*)

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Neural (Con't)

Species		Cell(s)	Enzyme(s)	Medium	Reference
Porcine	Porcine, adult, 60-100 kg	Superior cervical ganglia	Papain: 2 u/ml Collagenase: 0.12% Neutral Protease: 0.48%	HBSS	Si ML, Lee TJ.: Presynaptic alpha7-nicotinic acetylcholine receptors mediate nicotine-induced nitric oxidergic neurogenic vasodilation in porcine basilar arteries, *J Pharmacol Exp Ther 298*, 122, 2001 (*10052*)
Quail	Quail	Neural crest	Trypsin: 0.05%	MEM, HBSS	Sieber-Blum, M., and Cohen, A.: Clonal Analysis of Quail Neural Crest Cells: They are Pluripotent and Differentiate in Vitro in the Absence of Noncrest Cells, *Dev Biol 80*, 96, 1980 (*371*)
Rat	Rat, embryonic	Hypothalamic neurons and glias	Papain: 0.5 u/ml	DMEM	Yokosuka Makoto, Ohtani-Kaneko Ritsuko, Yamashita Kayoko, Muraoka Daisuke, Kuroda Yoichiro, Watanabe Chiho: Estrogen and environmental estrogenic chemicals exert developmental effects on rat hypothalamic neurons and glias, *Toxicol In Vitro 22*, 1-9, 2008 (*10346*)
	Rat, SD, embryos, 19-21 days gestation	Schwann, dorsal root ganglia	Trypsin: 0.25%	HBSS, CMF	Mithen, F., Reiker, M., and Birchem, R.: Effects of Ethanol on Rat Schwann Cell Proliferation and Myelination in Culture, *In Vitro Cell Dev Biol 26*, 129, 1990 (*430*)
	Rat (also bovine)	Heart Adrenal chromaffin Paraneurons	Trypsin: 0.06%	25mM HEPES buffered Locke's solution, CMF	Trifaro, J., Tang, R., and Novas, M.: Monolayer Co-Culture of Rat Heart Cells and Bovine Adrenal Chromaffin Paraneurons, *In Vitro Cell Dev Biol 26*, 335, 1990 (*438*)
	Rat, postnatal	Septal neurons	Papain: 0.05%	PBS, CMF	Hatanaka,H., Tsukui, H., Nihonmatsu, I.: Septal Cholinergic Neurons From Postnatal Rat Can Survive In The Dissociate Culture Conditions In The Presence Of Nerve Growth Factor, *Neurosci Lett 79*, 85, 1987 (*646*)
	Rat, Long Evans, 1-15 days	Neurons, visual cortex	Papain: 20 u/ml	BSS (see reference)	Huettner, J., and Baughman, R.: Primary Culture of Identified Neurons From the Visual Cortex of Postnatal Rats, *J Neurosci 6*, 3044, 1986 (*617*)
	Rat, embryo, 15 day	Dorsal horn neurons Spinal	Trypsin: 0.025%	Ham's F-12	Jahr, C. and Jessell, T.: Synaptic Transmission between Dorsal Root Ganglion and Dorsal Horn Neurons in Culture: Antagonism of Monosynaptic Excitatory Postsynaptic Potentials and Glutamate Excitation by Kynurenate, *J Neurosci 5*, 2281, 1985 (*614*)
	Rat, fetus, 18-20 day	Hippocampal neurons	Trypsin: 0.25%	HBSS, CMF	Bartlett, W. and Banker, G.: An Electron Microscopic Study of the Development of Axons and Dendrites by Hippocampal Neurons in Culture. I. Cells Which Develop Without Intercellular Contacts, *J Neurosci 4*, 1944, 1984 (*613*)
	Rat, postnatal	Ganglion, retina	Papain: 12.5 u/ml	HBSS w/5 mM HEPES	Leifer, D., Lipton, S., Barnstable, C., and Masland, R.: Monoclonal Antibody to Thy-1 Enhances Regeneration of Processes by Rat Retinal Ganglion Cells in Culture, *Science 224*, 303, 1984 (*667*)
	Rat, SD, pups	Retina	Trypsin: 0.25%	Ham's F-12	Sarthy PV, Curtis BM, and Catterall WA.: Retrograde Labeling, Enrichment, and Characerization of Retinal Ganglion Cells from the Neonatal Rat, *J Neurosci 3 (12)*, 2532, 1983 (*1199*)
	Rat, W/FU, 5-8 day	Neurons and glial	Trypsin: 0.25%	MEM (see reference)	Raff, M., Fields, K., Hakomori, S., Mirsky, R., Pruss, R., and Winter, J.: Cell-Type-Specific Markers for Distinguishing and Studying Neurons And the Major Classes of Glial Cells in Culture, *Brain Res 174*, 283, 1979 (*348*)
	Rat, fetus	Neurons, sympathetic	Trypsin: 0.25%	L-15 or HBSS, CMF	Wakshull, E., Johnson, M., Burton, H.: Postnatal Rat Sympathetic Neurons In Culture. 1. A Comparison With Embryonic Neurons, *J Neurophysiol 42*, 1410, 1979 (*716*)
	Rat, Wistar/Furth, newborn (also bovine)	Schwann	Trypsin: 0.25%	DMEM	Brockes, J., Fields, K., and Raff, M.: Studies on Cultured Rat Schwann Cells. I. Establishment of Purified Populations From Cultures of Peripheral Nerve, *Brain Res 165*, 105, 1979 (*991*)
	Rat, embryo	Neurons, cortical	Trypsin: 0.027%	MEM	Dichter, M.: Rat Cortical Neurons in Cell Culture: Culture Methods, Cell Morphology, Electrophysiology, and Synapse Formation, *Brain Res 149*, 279, 1978 (*346*)
	Rat, newborn	Neurons, sympathetic	Collagenase Type 1: 0.01%	Hank's solution, CF	Reichardt, L., Patterson, P.: Neurotransmitter Synthesis and Uptake by Isolated Sympathetic Neurones in Microcultures, *Nature 270*, 147, 1977 (*642*)
	Rat, neonatal	Neurons, superior cervical ganglia	Trypsin: 0.1%	Basal L-15 medium	Mains, R., and Patterson, P.: Primary Cultures of Dissociated Sympathetic Neurons I. Establishment of Long-Term Growth in Culture and Studies of Differentiated Properties, *J Cell Biol 59*, 329, 1973 (*587*)
Ovine	Lamb (also calf)	Oligodendroglia Neural	Trypsin: 0.1%	(see reference)	Poduslo, S., Miller, K., and McKhann, G.: Metabolic Properties of Maintained Oligodendroglia Purified from Brain, *J Biol Chem 253*, 1592, 1978 (*552*)
Salamander	Salamander (*A.tigrinum*)	Photoreceptors, retina	Papain: 0.05%	(see reference)	Bader, C., MacLeish, P., and Schwartz, E.: Responses to Light of Solitary Rod Photoreceptors Isolated From Tiger Salamander Retina, *Proc Natl Acad Sci U S A 75*, 3507, 1978 (*652*)
Shellfish	Snails (*Helisoma trivolis*), albino, adult	Buccal ganglia; SLT muscle	Trypsin: 0.2%	DMEM	Zoran, M., Doyle, R. and Haydon, P.: Target Contact Regulates the Calcium Responsiveness of the Secretory Machinery During Synaptogenesis, *Neuron 6*, 145, 1991 (*691*)
	Pond snail (*Helisoma*) albino, adult	Somata, buccal ganglia	Trypsin: 0.2%	Antibiotic saline, Leibowitz 50%	Haydon, P.: The Formation of Chemical Synapses Between Cell-Cultured Neuronal Somata, *J Neurosci 8*, 1032, 1988 (*620*)
	Helisoma trivovlis	Buccal ganglia	Trypsin: 0.2%	L-15 medium	Cohan, C., Haydon, P., and Kater, S.: Single Channel Activity Differs in Growing and Nongrowing Growth Cones of Isolated Identified Neurons of Helisoma, *J Neurosci Res 13*, 285, 1985 (*609*)
Turtle	Turtle (*Pseudemys scripta elegans*)	Retinal	Papain: 0.1% (13.5 u/mg)	Kreb's Ringer	Lam, D.: Biosynthesis of Acetylcholine in Turtle Photoreceptors, *Proc Natl Acad Sci U S A 69*, 1987, 1972 (*649*)

[CF=Calcium Free • CMF=Calcium Magnesium Free • BSS=Balanced Salt Solution]

Species		Cell(s)	Enzyme(s)	Medium	Reference
Bovine	Bovine (*Bos taurus*)	Ductal	Neutral Protease: 0.05%	EBSS	Sato, T., Sato, M., Hudson, E., and Jones, R.: Characterization of Bovine Pancreatic Ductal Cells Isolated by a Perfusion-Digestion Technique, *In Vitro 19*, 651, 1983 (*529*)
Canine	Canine	Buccal ganglia; SLT muscle	Trypsin: 0.2%	DMEM	Zoran, M., Doyle, R. and Haydon, P.: Target Contact Regulates the Calcium Responsiveness of the Secretory Machinery During Synaptogenesis, *Neuron 6*, 145, 1991 (*691*)
Fish	Fish, *Osphronemus gourami*, 3-month-old, 0.5 mg	Islets	Collagenase: 0.12 - 0.46 u/ml	RPMI 1640	Schrezenmeir, J., Laue, C., Sternheim,E., Wolbert, K., Darquy, S., Chicheportiche, D., Kirchgessner, J., and Reach, G.: Long-Term Function of Single-Cell Preparations of Piscine Principal Islets in Hollow Fibers, *Transplant Proc 24 (6)*, 2941, 1992 (*1221*)
Guinea-Pig	Guinea-pig, Hartley, albino, male, 350-400 g	Acinar	Soybean Trypsin Inhibitor: 0.01%	Kreb's Ringer	Gardner, J., Conlon, T., Klaeveman, H., Adams, T., and Ondetti, M.: Action of Cholecystokinin and Cholinergic Agents on Calcium Transport in Isolated Pancreatic Acinar Cells, *J Clin Invest 56*, 366, 1975 (*599*)
	Guinea-pig	Exocrine	Hyaluronidase: 0.15% - 0.2%	Kreb's Ringer	Amsterdam, J., and Jamieson, J.: Structural and Functional Characterization of Isolated Pancreatic Exocrine Cells, *Proc Natl Acad Sci U S A 69 (10)*, 3028, 1972 (*1151*)
Hamster	Hamster	Islets	Collagenase Type 4: 1.3% - 2.0%	HBSS	Feldman, J., and Chapman, B.: Preparation of Islets of Langerhans from Rabbits and Hamsters by the Collagenase Digestion Technique, *Acta Diabetol 12*, 208, 1975 (*686*)
Human	Human	Islets	Collagenase Type 4: 0.8%	HBSS	Izumi, R., Konishi, K., Ueno, K., Shimizu, K., Hirosawa, H., Takahashi, N., and Miyazaki, I.: Isolation of Human Pancreatic Islets from Cryopreserved Pancreas, *Transplant Proc XVII*, 383, 1985 (*689*)
	Human	Islets	Collagenase (1 or 4): 0.60%	HBSS	Gray, D., McShane, P., Grant, A., and Morris, P.: A Method for Isolation of Islets of Langerhans from the Human Pancreas, *Diabetes 33*, 1055, 1984 (*690*)
	Human, infant, age 1 day-1 year	Islets	Collagenase: 170-210 u/ml	HBSS	Sutherland, D., Matas, A., Steffes, M., and Najarian, J.: Infant Human Pancreas: A Potential Source of Islet Tissue for Transplantation, *Diabetes 25 (12)*, 1123, 1976 (*810*)
Monkey	Monkey, 3-5 Kg	Islets	Hyaluronidase: 0.05%	HBSS	Scharp, D., Murphy, J., Newton, W., Ballinger, W., and Lacy, P.: Application of an Improved Isolation Technique for Islet Transplantation in Primates and Rats, *Transplant Proc 7*, 739, 1975 (*688*)
Mouse	Mouse	Pancreatic islet	Collagenase Type 4: 0.2%	HBSS	Haefliger Jacques-Antoine, Tawadros Thomas, Meylan Laure, Gurun SabineLe, Roehrich Marc-Estienne, Martin David, Thorens Bernard, Waeber Gerard: The scaffold protein IB1/JIP-1 is a critical mediator of cytokine-induced apoptosis in pancreatic beta cells, *J Cell Sci 116*, 1463-9, 2003 (*10151*)
	Mouse	Islets	Collagenase Type 4: 0.2%	HBSS	Wu Yulian, Han Bing, Luo Hongyu, Roduit Raphael, Salcedo TheodoraW, Moore PaulA, Zhang Jun, Wu Jiangping: DcR3/TR6 effectively prevents islet primary nonfunction after transplantation, *Diabetes 52*, 2279-86, 2003 (*10288*)
	Mouse, BALB/c, 6-8 week-old, either sex	Acinar	Collagenase: 0.1%	Waymouth's MB	Kurup, S., and Bhonde, R.: Analysis and Optimization of Nutritional Set-up for Murine Pancreatic Acinar Cells, *JOP 3 (1)*, 8, 2002 (*1070*)
	Mouse	Acinar	Collagenase: 100 u/ml	HEPES	Jauch, P., Peterson, O., and Lauger, P.: Electrogenic Properties of the Na-Alanine Cotransporter in Acinar Cells, *J Membr Biol 94*, 99, 1986 (*605*)
	Mouse (C57BL/6J-ob/ob), 9-12 wks, male	Islets	Hyaluronidase: 0.5%	Kreb's Ringer bicarbonate buffer	Dalpe-Scott, M., Heick, H., and Begin-Heick, N.: Secretion in the Obese (ob/ob) Mouse. The Effect of Oxytetracycline on Insulin Release, *Diabetes 32*, 932, 1983 (*687*)
Porcine	Porcine, juvenile	Islets	Collagenase: see reference	Univ of Wisconsin solution	Van der Burg Michael P M, Graham John M: Iodixanol Density Gradient Preparation in University of Wisconsin solution for porcine islet purification, *ScientificWorldJournal 3*, 1154-9, 2003 (*10286*)
	Porcine, 3 month, 15-20 kg	Acinar	Collagenase Type 3: 200 u/ml	RPMI 1640	Zhao, X., Han, J., and Tang, C.: Primary Culture of Porcine Pancreatic Acinar Cells, *JOP 2 (2)*, 78, 2001 (*768*)
	Porcine	Acinar	Collagenase: 100 u/ml	Saline	Iwatsuki, N., and Peterson, O.: Action of Tetraethylammonium on Calcium-Activated Potassim Channels in Pig Pancreatic Acinar Cells Studied by Patch-Clamp Single-Channel and Whole-Cell Current Recording, *J Membr Biol 86*, 139, 1985 (*604*)
Rabbit	Rabbit, New Zealand white, Male/Female, 2-3 Kg	Acinar	Hyaluronidase: 0.2%	Kreb's Ringer bicarbonate buffer	Renckens, B., Schrijen, J., Swarts, H., DePont, J., and Bonting, S.: Role of Calcium in Exocrine Pancreatic Secretion. IV. Calcium Movements in Isolated Acinar Cells of Rabbit Pancreas, *Biochim Biophys Acta 544*, 338, 1978 (*321*)
	Rabbit, also hamster	Islets	Collagenase Type 4: 1.3% - 2.0%	HBSS	Feldman, J., and Chapman, B.: Preparation of Islets of Langerhans from Rabbits and Hamsters by the Collagenase Digestion Technique, *Acta Diabetol 12*, 208, 1975 (*686*)
Rat	Rat, Wistar, male, 250-400g	Pancreatic islets	Collagenase: 0.75%	RPMI 1640	Tian XH, Xue WJ, Ding XM, Pang XL, Teng Y, Tian PX, and Feng XS: Small intestinal submucosa improves islet survival and function during in vitro culture, *World J Gastroenterol 11*, 7378, 2005 (*10008*)
	Rat, 250-350 g	Pancreatic islet	Collagenase Type 4: 0.2%	HBSS	Haefliger Jacques-Antoine, Tawadros Thomas, Meylan Laure, Gurun SabineLe, Roehrich Marc-Estienne, Martin David, Thorens Bernard, Waeber Gerard: The scaffold protein IB1/JIP-1 is a critical mediator of cytokine-induced apoptosis in pancreatic beta cells, *J Cell Sci 116*, 1463-9, 2003 (*10151*)
	Rat	Pancreatic acini	CLSPA: 30 u/ml Collagenase Type 4: 30 u/ml Soybean Trypsin Inhibitor: 0.01%	M199	Blinman TA, Gukovsky I, Mouria M, Zaninovic V, Livingston E, Pandol SJ, Gukovskaya AS: Activation of pancreatic acinar cells on isolation from tissue: cytokine upregulation via p38 MAP kinase, *Am J Physiol Cell Physiol 279*, C1993-2003, 2000 (*10133*)
	Rat, SD, male, 40 - 100 g	Acinar	Hyaluronidase: 462 u/ml	Ham's F12	Hirschi, K., Kenny, S., Justice, J., Brannon, P.: Effects of Secretin And Caerulein On Enzymes Of Cultured Pancreatic Acinar Cells, *In Vitro Cell Dev Biol 27*, 660, 1991 (*472*)
	Rat, SD, male, 100 - 150 g	Parotid acinar	Trypsin: 0.001%	F12 medium	Yeh, C., Mertz, P., Oliver, C., Baum, B., and Kousvelari, E.: Cellular Characteristics of Long-Term Cultured Rat Parotid Acinar Cells, *In Vitro Cell Dev Biol 27*, 707, 1991 (*473*)

[CF=Calcium Free • CMF=Calcium Magnesium Free • BSS=Balanced Salt Solution]

Pancreas (Con't)

Pancreas (Con't)

Species		Cell(s)	Enzyme(s)	Medium	Reference
Rat	Rat, Wistar, male	Parotid acinar	Trypsin: 0.02%	Solution B (see reference)	Foskett, J., Roifman, C. and Wong, D.: Activation of Calcium Oscillations by Thapsigargin in Parotid Acinar Cells, *J Biol Chem 266*, 2778, 1991 (*573*)
	Rat, SD, both sexes, 6-20 wks old	Interlobular ducts	Papain: 25 u/ml	DMEM/Ham's F-12	Githens, S., Schexnayder, J., Desai, K., and Patke, C.: Rat Pancreatic Interlobular Duct Epithelium: Isolation and Culture in Collagen Gel, *In Vitro Cell Dev Biol 25 (8)*, 679, 1989 (*790*)
	Rat, SD, male, 150 - 200 g	Acinar	Soybean Trypsin Inhibitor: 0.01%	HEPES	Menozzi, D., Sato, S., Jensen, R., and Gardner, J.: Cyclic GMP Does Not Inhibit Protein Kinase C-Mediated Enzyme Secretion in Rat Pancreatic Acini, *J Biol Chem 264*, 995, 1989 (*565*)
	Rat, Wistar, male, 250 - 350 g	Acinar, parotid	Hyaluronidase: 0.015%	Earle's MEM	Melvin, J., Kawaguchi, M., Baum, B., and Turner, R.: A Muscarinic Agonist-Stimulated Chloride Efflux Pathway is Associated With Fluid Secretion in Rat Parotid Acinar Cells, *Biochem Biophys Res Commun 145*, 754, 1987 (*308*)
	Rat, SD, male, 50 - 125 g	Acinar Exorbital lacrimal, parotid, pancreas	Trypsin: 0.01%	HBSS, CMF	Oliver, C., Waters, J., Tolbert, C., and Kleinman, H.: Growth of Exocrine Acinar Cells on a Reconstituted Basement Membrane Gel, *In Vitro Cell Dev Biol 23*, 465, 1987 (*421*)
	Rat, SD, male, 42 - 48 day, 175 - 200 g	Acinar, submandibular gland	Hyaluronidase: 0.1 %	HBSS, CF	Quissell, D., Redman, R., and Mark, M.: Short-Term Primary Culture of Acinar-Intercalated Duct Complexes From Rat Submandibular Glands, *In Vitro Cell Dev Biol 22*, 469, 1986 (*419*)
	Rat, SD, 125 - 350 g	Duct	Trypsin: 0.01%	HBSS	Githens, S., Holmquist, D., Whelan, J., and Ruby, J.: Ducts of the Rat Pancreas in Agarose Matrix Culture, *In Vitro 16*, 797, 1980 (*510*)
	Rat, SD, male, 250 - 350 g (also mouse, white, Swiss, male, 20 - 24 g)	Acinar	Hyaluronidase: 0.18%	Kreb's Henseleit bicarbonate buffer	Williams, J., Korc, M., and Dormer, R.: Action of Secretagogues on a New Preparation of Functionally Intact, Isolated Pancreatic Acini, *Am J Physiol 235*, 517, 1978 (*288*)
	Rat, Wistar, male, 200 - 300 g	Islets	Collagenase Type 4: 0.5%	HBSS	Shibata, A., Ludvigsen, C., Naber, S., McKaneil M., and Lacy, P.: Standardization of a Digestion-Filtration Method for Isolation of Islets, *Diabetes 25*, 667, 1976 (*677*)
	Rat, neonate	Islets	Trypsin: 0.05%	Puck's saline buffered w/ EDTA 0.02%	Leonard, R., Lazarow, A., and Hegre, O.: Pancreatic Islet Transplantation in the Rat, *Diabetes 22*, 413, 1973 (*684*)
	Rat, Lewis, inbred	Islets	Collagenase: 0.5%	HBSS	Ballinger, W., and Lacy, P.: Transplantation of Intact Pancreatic Islets in Rats, *Surgery 72 (2)*, 175, 1972 (*1119*)
	Rat, Wistar, albino, male, 400 - 500 g	Islets	Collagenase Type 4: 1.0% - 1.2%	HBSS	Lacy, P., and Kostianovsky, M.: Method for the Isolation of Intact Islets of Langerhans from the Rat Pancreas, *Diabetes 16*, 35, 1967 (*685*)
	Rat	Ascites hepatoma	Trypsin: 0.1%	Phosphate buffer (see reference)	Essner, E.: Experiments on an Ascites Hepatoma. I. Enzymatic Digestion and Alkaline Degradation of the Cementing Substance and Separation of Cells, in Tumor Islands, *Exp Cell Res 7*, 430, 1954 (*403*)

Parotid

Parotid

Species		Cell(s)	Enzyme(s)	Medium	Reference
Rat	Rat, Wistar, male, 130 g	Acinar	Collagenase: 75 u/ml Hyaluronidase: 153 u/ml	RPMI 1640	Looms, D., Dissing, S., Tritsaris, K., Pedersen, A. and Nauntofte, B.: Adrenoceptor-Activated Nitric Oxide Synthesis in Salivary Acinar Cells., *Adv Dent Res Vol. 14*, 62, 2000 (*10629*)
	Rat, SD, male, 100-150 g	Parotid acinar	Trypsin: 0.001%	F12 medium	Yeh, C., Mertz, P., Oliver, C., Baum, B., and Kousvelari, E.: Cellular Characteristics of Long-Term Cultured Rat Parotid Acinar Cells, *In Vitro Cell Dev Biol 27*, 707, 1991 (*473*)
	Rat, Wistar, male	Parotid acinar	Trypsin: 0.02%	Solution B (see reference)	Foskett, J., Roifman, C. and Wong, D.: Activation of Calcium Oscillations by Thapsigargin in Parotid Acinar Cells, *J Biol Chem 266*, 2778, 1991 (*573*)
	Rat	Parotid	Hyaluronidase: 0.025%	HBSS with 20mM HEPES	Takuma, T. and Ichida, T.: Amylase Secretion From Saponin-Permeabilized Parotid Cells Evoked by Cyclic AMP, *J Invest Dermatol 103*, 95, 1988 (*676*)
	Rat, Wistar, male, 250 - 350 g	Acinar, parotid	Hyaluronidase: 0.015%	Earle's MEM	Melvin, J., Kawaguchi, M., Baum, B., and Turner, R.: A Muscarinic Agonist-Stimulated Chloride Efflux Pathway is Associated With Fluid Secretion in Rat Parotid Acinar Cells, *Biochem Biophys Res Commun 145*, 754, 1987 (*308*)
	Rat, SD, male, 50 - 125 g	Acinar Exorbital lacrimal, parotid, pancreas	Trypsin: 0.01%	HBSS, CMF	Oliver, C., Waters, J., Tolbert, C., and Kleinman, H.: Growth of Exocrine Acinar Cells on a Reconstituted Basement Membrane Gel, *In Vitro Cell Dev Biol 23*, 465, 1987 (*421*)

Pituitary

Pituitary

Species		Cell(s)	Enzyme(s)	Medium	Reference
Bovine	Bovine	Pituitary	Collagenase: 0.1%	EBSS, CMF	Mason, W. and Ingram, C.: Techniques for Studying the Role of Electrical Activity in Control of Secretion by Normal Anterior Pituitary Cells, *Vol. 124*, 207, 1986 (*632*)
	Bovine	Follicular, anterior pituitary and pars tuberalis	Deoxyribonuclease I: 200 µg/ml	HBSS, CMF	Ferrara, N., Goldsmith, P., Fujii, D., and Weiner, R.: Culture and Characterization of Follicular Cells of the Bovine Anterior Pituitary and Pars Tuberalis, *Vol. 124*, 245, 1986 (*633*)
	Calf, male, 1-6 week old	Pitituary	Hyaluronidase: 0.1%	DMEM	Ridgway, E., Klibanski, A., Marorana, M., Milbury, P., Kieffer, J., and Chin, W.: The Effect of Somatostatin on the Release of Thyrotropin and its Subunits from Bovine Anterior Pituitary Cells *in Vitro*, *Endocrinology 112 (6)*, 1937, 1983 (*1026*)
Mouse	Mouse, male	Pituitary	Collagenase: 0.4% Hyaluronidase: 0.1% Trypsin: 0.3%	DMEM/Han's F12	Steveson Tami C, Ciccotosto Giuseppe D, Ma Xin-Ming, Mueller Gregory P, Mains Richard E, Eipper Betty A: Menkes protein contributes to the function of peptidylglycine alpha-amidating monooxygenase, *Endocrinology 144*, 188-200, 2003 (*10241*)
Ovine	Ovine, adult	Somatotropes	Collagenase Type 1: 0.3% Hyaluronidase: Collagenase Type 1:	Medium 199	Xu Ruwei, Wang Qinling, Yan Ming, Hernandez Maria, Gong Changhong, Boon WahChin, Murata Yoko, Ueta Yoichi, Chen Chen: Orexin-A augments voltage-gated Ca2+ currents and synergistically increases growth hormone (GH) secretion with GH-releasing hormone in primary cultured ovine somatotropes, *Endocrinology 143*, 4609-19, 2002 (*10246*)

[CF=Calcium Free • CMF=Calcium Magnesium Free • BSS=Balanced Salt Solution]

Pituitary (Con't)					**Pituitary** (Con't)
Species		Cell(s)	Enzyme(s)	Medium	Reference
Rat	Rat, SD, female, 200 - 250 g	Anterior pituitary gland	Trypsin: 0.1%	EBSS, CMF	D'Emden, M. and Wark, J.: Culture Requirements for Optimal Expression of 1,25-Dihydroxyvitamin D3-Enhanced Thyrotropin Secretion, *In Vitro Cell Dev Biol 27*, 197, 1991 (*459*)
Prostate					**Prostate**
Species		Cell(s)	Enzyme(s)	Medium	Reference
Human	Human, 52-56 yr	Prostatic stromal cells	Collagenase Type 1: 0.2%	DMEM/F-12	Le Hanh, Arnold Julia T, McFann Kimberly K, Blackman Marc R: DHT and testosterone, but not DHEA or E2, differentially modulate IGF-I, IGFBP-2, and IGFBP-3 in human prostatic stromal cells, *Am J Physiol/ Endo 290*, E952-60, 2006 (*10126*)
	Human	Prostate stromal cells	Collagenase Type 1: 0.1%	RPMI 1640	Nakashiro Koh-Ichi, Hara Shingo, Shinohara Yuji, Oyasu Miho, Kawamata Hitoshi, Shintani Satoru, Hamakawa Hiroyuki, Oyasu Ryoichi: Phenotypic switch from paracrine to autocrine role of hepatocyte growth factor in an androgen-independent human prostatic carcinoma cell line, CWR22R, *Am J Pathol 165*, 533-40, 2004 (*10163*)
	Human, fetal	Prostatic fibroblasts	Collagenase Type 1: 0.125%	DMEM/F12	Levine AC, Liu XH, Greenberg PD, Eliashvili M, Schiff JD, Aaronson SA, Holland JF, Kirschenbaum A: Androgens induce the expression of vascular endothelial growth factor in human fetal prostatic fibroblasts, *Endocrinology 139*, 4672-8, 1998 (*10124*)
Mouse	Mouse, 6-8 week	Prostate epithelial/stem	Collagenase Type 2: 0.5% Trypsin: 0.05%	HBSS	Burger, P., Gupta, R., Xiong, X., Ontiveros, C., Salm, S., Moscatelli, D. and Wilson, E.: High Aldehyde Dehydrogenase Activity: A Novel Functional Marker of Murine Prostate Stem/Progenitor Cells., *Stem Cells 27*, 2220-8, 2009 (*10488*)
	Mouse, 2 week	Prostatic epithelial	Collagenase Type 3: 170 u/ml	DMEM	Imamov Otabek, Morani Andrea, Shim Gil-Jin, Omoto Yoko, Thulin-Andersson Christina, Warner Margaret, Gustafsson Jan-Ake: Estrogen receptor beta regulates epithelial cellular differentiation in the mouse ventral prostate, *Proc Natl Acad Sci U S A 101*, 9375-80, 2004 (*10224*)
	Mouse, male	Prostatic stem	Collagenase Type 1: 170 u/ml	DMEM	Dubey P, Wu H, Reiter RE, Witte ON: Alternative pathways to prostate carcinoma activate prostate stem cell antigen expression, *Cancer Res 61*, 3256-61, 2001 (*10229*)
Reproductive					**Reproductive**
Species		Cell(s)	Enzyme(s)	Medium	Reference
Bovine	Bovine	Corpus leuteal cells	Collagenase Type 4: 420 u/ml	M-199	Levy N, Gordin M, Mamluk R, Yanagisawa M, Smith M F, Hampton J H, Meidan R: Distinct cellular localization and regulation of endothelin-1 and endothelin-converting enzyme-1 expression in the bovine corpus luteum: implications for luteolysis, *Endocrinology 142*, 5254-60, 2001 (*10169*)
	Bovine (also porcine, human)	Interna & corpus luteum Endometrium Ovarian Uterine	Pronase: 0.1%	Moscona's BSS	Marcus, G., Connor, L., Domingo, M., Tsang, B., Downey, B., and Ainsworth, L.: Enzymatic Dissociation of Ovarian and Uterine Tissues, *Endocr Res 10*, 151, 1984 (*372*)
Chicken	Chicken, Gallus Domesticus,20-30 week	Primary follicles	Trypsin: 0.15% Collagenase Type 1: 0.125%	Dulbecco's phosphate buffered saline	Du Meihong, Han Haitang, Jiang Bin, Zhao Chen, Qian Changsong, Shen Haiyan, Xu Yan, Li Zandong: An efficient isolation method for domestic hen (Gallus domesticus) ovarian primary follicles, *J Reprod Dev 52*, 569-76, 2006 (*10301*)
Frog	Xenopus laevis, female	Oocytes	Collagenase Type 1: 0.2%	(see reference)	Pannaccione Anna, Castaldo Pasqualina, Ficker Eckhard, Annunziato Lucio, Taglialatela Maurizio: Histidines 578 and 587 in the S5-S6 linker of the human Ether-a-gogo Related Gene-1 K+ channels confer sensitivity to reactive oxygen species, *J Biol Chem 277*, 8912-9, 2002 (*10166*)
	Xenopus laevis, mature female	Oocytes	Collagenase: 0.2%	CF Medium	Moriarty, T., Gillo, B., Carty, D., Premont, R., Landau, E., Iyengar, R.: Beta gamma Subunits of GTP-Binding Proteins Inhibit Muscarinic Receptor Stimulation of Phospholipase C, *Proc Natl Acad Sci U S A 85*, 8865, 1988 (*661*)
Hamster	Hamster, Chinese	Ovary	Trypsin: 5%	Dialyzed fetal calf serum, 10% and 0.5M Methotrexate	Wallis, R., and Drickamer, K.: Molecular Determinants of Oligomer Formation and Complement Fixation in Mannose-Binding Proteins, *J Biol Chem 274 (6)*, 3580, 1999 (*1125*)
Human	Human, female	Decidual	Collagenase: 0.25% Deoxyribonuclease I: 6.25 u/ml	DMEM/F12	Lockwood, C., Arcuri, F., Toti, P., Felice, C., Krikun, G., Guller, S., Buchwalder, L. and Schatz, F.: Tumor Necrosis Factor-Alpha and Interleukin-1 Beta Regulate Interleukin-8 Expression in Third Trimester Decidual Cells: Implications for the Genesis of Chorioamnionitis., *Am J Pathol 169*, 1294-302, 2006 (*10353*)
	Human	Sertoli cells	Trypsin: 2.5% Collagenase Type 1: 2% Hyaluronidase: 1%	DMEM/F-12	Teng Yan, Xue Wu-jun, Ding Xiao-ming, Feng Xin-shun, Xiang He-li, Jiang Ya-zhuo, Tian Pu-xun: Isolation and culture of adult Sertoli cells and their effects on the function of co-cultured allogeneic islets in vitro, *Chin Med J (Engl) 118*, 1857-62, 2005 (*10322*)
	Human, female, 34-51 yr	Endometrial epithelial and stromal cells	Collagenase Type 3: 0.03% Deoxyribonuclease I: 0.004%	DMEM/F-12	Chan Rachel W S, Schwab Kjiana E, Gargett Caroline E: Clonogenicity of human endometrial epithelial and stromal cells, *Biol Reprod 70*, 1738-50, 2004 (*10137*)
	Human, 20-40 yr	Endometrium epithelial and stromal cells	Collagenase Type 1: 0.2%	HBSS	Arnold, J., Kaufman, D., Seppala, M., and Lessey, B.: Endometrial stromal cells regulate epithelial cell growth in vitro: a new co-culture model, *Hum Reprod 16*, 836, 2001 (*9820*)
	Human	Chorionic villi	Trypsin: see reference Collagenase Type 3: 100 u/ml	HBSS	Yusuf RZ, Naeem R: Cytogenetic studies of spontaneous miscarriages: a seven year study to compare significance of primary vs. secondary culture methods for assessment of fetal karyotype yield and maternal cell contamination, *Early Pregnancy 5*, 121-31, 2001 (*10290*)
	Human	Endometrial endothelial cells	Collagenase Type 1: 0.2%	McCoys medium	Nikitenko LL, MacKenzie IZ,Rees MC,Bicknell R: Adrenomedullin is an autocrine regulator of endothelial growth in human endometrium, *Mol Hum Reprod 6*, 811, 2000 (*10029*)

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Reproductive (Con't)

Species		Cell(s)	Enzyme(s)	Medium	Reference
Human	Human	Trophoblasts, placental	Trypsin: 0.25%	EBSS, CMF	Branchaud, C.L., Goodyer, C.G., Guyda, H.J. and Lefebvre, Y.: A Serum-Free System for Culturing Human Placental Trophoblasts, *In Vitro Cell Dev Biol 26*, 865, 1990 (*453*)
	Human	Trophoblasts, placental	Trypsin: 0.25%	PBS	Jie, Z., Fey, S., Hager, H., Hollsberg, P., Ebbesen, P., and Larsen, P.: Markers For Human Placental Trophoblasts in Two-Dimensional Gel Electrophoresis, *In Vitro Cell Dev Biol 26*, 937, 1990 (*455*)
	Human	Chorionic, placental	Deoxyribonuclease I: 0.003%	HBSS	Egan, D., Grzegorczyk, V., Tricarico, K., Rueter, A.H., Olleman, W., and Marcotte, P.: Human Placental Chorionic Renin: Production, Purification and Characterization, *Biochim Biophys Acta 965*, 68, 1988 (*335*)
	Human (also porcine, bovine)	Interna & corpus luteum Endometrium Ovarian Uterine	Pronase: 0.1%	Moscona's BSS	Marcus, G., Connor, L., Domingo, M., Tsang, B., Downey, B., and Ainsworth, L.: Enzymatic Dissociation of Ovarian and Uterine Tissues, *Endocr Res 10*, 151, 1984 (*372*)
	Human, female, 27-49 years	Epithelial Ovary	Trypsin: 0.125%	HBSS, CMF	Auersperg, N., Siemens, C.H., and Myrdal, S.E.: Human Ovarian Surface Epithelium In Primary Culture, *In Vitro 20*, 743, 1984 (*535*)
	Human, female, 27 years	Smooth muscle, uterine	Trypsin: 0.05%	EBSS	Rifas, L., Fant, J., Makman, M., and Seifter, S.: The Characterization of Human Uterine Smooth Muscle Cells in Culture, *Cell Tissue Res 196*, 385, 1979 (*355*)
Mouse	Mouse, 6 day	Sertoli	Collagenase Type 2: 500 u/ml Hyaluronidase: 0.1% Deoxyribonuclease I: .0005%	DMEM	Nalbandian Angele, Dettin Luis, Dym Martin, Ravindranath Neelakanta: Expression of vascular endothelial growth factor receptors during male germ cell differentiation in the mouse, *Biol Reprod 69*, 985-94, 2003 (*10162*)
	Mouse, adult, neonatal and fetal	Testicular cells	Collagenase Type 1: 0.1%	DMEM/F-12	O'Shaughnessy PJ, Fleming LM, Jackson G, Hochgeschwender U, Reed P, Baker PJ: Adrenocorticotropic hormone directly stimulates testosterone production by the fetal and neonatal mouse testis, *Endocrinology 144*, 3279-84, 2003 (*10165*)
	Mouse, female,6-8 weeks	Uterine	Trypsin: 0.2%	HBSS	Ghosh, D., Danielson, K., Alston, J., Heyner, S.: Functional Differentiation of Mouse Uterine Epithelial Cells Grown On Collagen Gels Or Reconstituted Basement Membranes, *In Vitro Cell Dev Biol 27*, 713, 1991 (*474*)
	Mouse, CF1, female	Cumulus, one-cell embryos	Hyaluronidase: 0.1%	PBS, CMF	Spindle, A.: In vitro Development of One-Cell Embryos from Outbred Mice: Influence of Culture Medium Composition, *In Vitro Cell Dev Biol 26*, 151, 1990 (*424*)
	Mouse BALB/cCRGL, male, 2-3 months	Epithelial, prostate gland	Hyaluronidase: 0.1%	Medium 199	Turner, T., Bern, H., Young,P., and Cunha, G.: Serum-Free Culture of Enriched Mouse Anterior and Ventral Prostatic Epithelial Cells in Collagen Gel, *In Vitro Cell Dev Biol 26*, 722, 1990 (*449*)
	Mouse C57B/T, fetus, 16-17 days old	Prostate	Trypsin: 1.0%	HBSS/ DMEM	Thompson, T.C., Southgate, J., Kitchener, G., and Land, H.: Multistage Carcinogenesis Induced by ras and myc Oncogenes in a Reconstituted Organ, *Cell 56*, 917, 1989 (*360*)
	Mouse, male, 10-13 wk	Leydig Testis	Deoxyribonuclease I: 0.001%	Medium 199 w/ BSA	Stalvcey, J. and Payne, A.: Luteinizing Hormone Receptors and Testosterone Production in Whole Testes and Purified Leydig Cells from the Mouse: Differences among Inbred Strains, *Endocrinology 112*, 1696, 1983 (*376*)
	Mouse BALB/cCrgl, female, 50 - 60 days	Epithelial, vagina	Collagenase Type 3: 0.1%	HBSS	Iguchi, T., Uchima, F.A., Ostrander, P., and Bern, H.: Growth of Normal Mouse Vaginal Epithelial Cells in and on Collagen Gels, *Proc Natl Acad Sci U S A 80*, 3743, 1983 (*655*)
Ovine	Sheep	Epithelial	Collagenase: 125 - 190 u/ml	DMEM	Salamonsen, L., Sum O, W., Doughton, B., and Findlay, J.: The Effects of Estrogen and Progesterone *In Vivo* on Protein Synthesis and Secretion by Cultured Epithelial Cells from Sheep Endometrium, *Endocrinology 117 (5)*, 2148, 1985 (*1193*)
Porcine	Porcine (also bovine, human)	Interna & corpus luteum Endometrium Ovarian Uterine	Pronase: 0.1%	Moscona's BSS	Marcus, G., Connor, L., Domingo, M., Tsang, B., Downey, B., and Ainsworth, L.: Enzymatic Dissociation of Ovarian and Uterine Tissues, *Endocr Res 10*, 151, 1984 (*372*)
	Porcine, 3-4 week	Leydig Testis	Trypsin: 0.0003%	Lebovitz L-15 Medium	Mather, J., Saez, J., and Haour, F.: Regulaton of Gonadotropin Receptors and Steroidogenesis in Cultured Porcine Leydig Cells, *Endocrinology 110*, 933, 1982 (*374*)
Rabbit	Rabbit, New Zealand white, adult, female (nonpregnant), 3-4 kg	Myocytes, uterine	Deoxyribonuclease I: 200 µg/ml	HBSS-HEPES buffer	Phillippe, M., Saunders, T., and Bangalore, S.: Alpha-1,Alpha-2,and Beta Adrenergic Signal Transduction in Cultured Uterine Myocytes, *In Vitro Cell Dev Biol 26*, 369, 1990 (*439*)
	Rabbit, New Zealand, mature, female, 4-5 Kg	Ovarian surface epithelial and peritoneal mesothelial	Collagenase Type 1: 300 IU/ml (280 IU/mg)	HBSS, CMF	Piquette, G., and Timms, B.: Isolation and Characterization of Rabbit Ovarian Surface Epithelium, Granulosa Cells, and Peritoneal Mesothelium in Primary Culture, *In Vitro Cell Dev Biol 26*, 471, 1990 (*443*)
	Rabbit, New Zealand white estrous, female, 4-5 months	Mesothelial and surface epithelial Ovaries	Trypsin: 0.125%-0.5%	Medium 199	Nicosia, S., Johnson, J., and Streibel, E.: Isolation and Ultrastructure of Rabbit Ovarian Mesothelium(Surface Epithelium), *Int J Gynecol Pathol 3*, 348, 1984 (*542*)
Rat	Rat, SD, 350-450 g	Leydig cells	Collagenase: .05-0.1%	Medium 199	Sharma RS, Pal PC, Rajalakshmi M.: Isolation and Culture of Leydig Cells from Adult Rats, *Indian J Clinical Biochem 21*, 27, 2006 (*10026*)
	Rat, SD, adult, male, 8 - 10 week	Seminiferous tubules	Trypsin: 0.05%	Krebs-Ringer bicarbonate buffer (see reference)	Abou-Haila, A., and Tulsiani, D.: Acid Glycohydrolases in Rat Spermatocytes, Spermatids and Spermatozoa: Enzyme Activities, Biosynthesis and Immunolocalization, *Biol Proced Online 3 (1)*, 35, 2001 (*1074*)
	Rat, SD, male, 90 days	Sertoli	Hyaluronidase: 0.1%	DMEM	Onoda, M. and Djakiew, D.: Pachytene Spermatocyte Protein(s) Stimulate Sertoli Cells Grown in Bicameral Chambers: Dose-Dependent Secretion of Ceruloplasmin, Sulfated Glycoprotein-1, Sulfated Glycoprotein-2, and Transferrin, *In Vitro Cell Dev Biol 27*, 215, 1991 (*460*)
	Rat, SD, male	Leydig	Trypsin: 0.02%	DMEM	Abayasekara, D., Kurlak, L., Band, A., Sullivan, M., and Cooke, B.: Effect of Cell Purity, Cell Concentration, and Incubation Conditions on Rat Testis Leydig Cell Steroidogenesis, *In Vitro Cell Dev Biol 27*, 253, 1991 (*461*)

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Reproductive (Con't)

Reproductive (Con't)

Species		Cell(s)	Enzyme(s)	Medium	Reference
Rat	Rat, neonatal	Uterine	Trypsin: 1%	HBSS	Branham, W., Lyn-Cook, B., Andrews, A., McDaniel, M., Sheehan, D.: Growth of Neonatal Rat Uterine Luminal Epithelium on Extracellular Matrix, *In Vitro Cell Dev Biol 27*, 442, 1991 (*465*)
	Rat, SD, male, 120-160 g	Leydig Adrenal	Collagenase Type 2: 0.03% (adrenal)	Krebs Ringer bicarbonate buffer	Ng, T. and Liu, W.: Toxic Effect of Heavy Metals on Cells Isolated from the Rat Adrenal and Testis, *In Vitro Cell Dev Biol 26*, 24, 1990 (*435*)
	Rat, Wistar, adult	Uterine	Trypsin: 0.5%	PBS	Pampfer, S., Vanderheyden, I., Michiels, B., and DeHertogh, R.: Co-Culture of Two-Cell Rat Embryos on Cell Monolayers, *In Vitro Cell Dev Biol 26*, 944, 1990 (*456*)
	Rat, immature	Luteal, ovaries	Collagenase: 0.3%	McCoy's	Rajan, V. and Menon, K.: Differential Uptake and Metabolism of Free and Esterified Cholesterol from High-density Lipoproteins in the Ovary, *Biochim Biophys Acta 959*, 206, 1988 (*330*)
	Rat, male, 20 day	Sertoli	Trypsin: 0.15%	(see reference)	Skinner, M., Fetterolf, P., and Anthony, C.: Purification of a Paracrine Factor, P-Mod-S, Produced by Testicular Peritubular Cells That Modulates Sertoli Cell Function, *J Biol Chem 263*, 2884, 1988 (*561*)
	Rat, SD, female, 21 day	Luteal, ovaries	Deoxyribonuclease I: 0.0004%	McCoy's	Rajan, V. and Menon, K.: Involvement of Microtubules in Lipoprotein Degradation and Utilization for Steroidogenesis in Cultured Rat Luteal Cells, *Endocrinology 117*, 2408, 1985 (*382*)
	Rat, SD, male, 10 day	Sertoli	Trypsin: 0.025%	DMEM	Hadley, M., Byers, S., Suarez-Quian, C., Kleinman, H., and Dym, M.: Extracellular Matrix Regulates Seratoli Cell Differentiation, Testicular Cord Formation and Germ Cell Development In Vitro, *J Cell Biol 101*, 1511, 1985 (*575*)
	Rat, SD, adult,male, 50-70 days	Testicular	Deoxyribonuclease I: 10 µg/ml	HEPES	Hsueh, A., Bambino, T., Zhuang, L., Welsh, T., and Ling, N.: Mechanism of the Direct Action of Gonadotropin Releasing Hormone and Its Antagonist on Androgen Biosynthesis by Cultured Rat Testicular Cells, *Endocrinology 112*, 1653, 1983 (*375*)
	Rat, SD, female, pseudopregnant, 21 day	Luteal, ovaries	Hyaluronidase: 0.1%	EBSS	Rajendran, K., Hwang, J., and Menon, K.: Binding, Degradation an Utilization of Plasma High Density and Low Density Lipoproteins for Progesterone Production In Cultured Rat Luteal Cells, *Endocrinology 112*, 1746, 1983 (*377*)
	Rat, SD, 4-6 day, male	Sertolli, seminiforous tubules	Collagenase: 0.03%	Serum-free medium	Rich, K., Bardin, C., Gunsalus, G., and Mather, J.: Age-Dependent Pattern of Androgen-Binding Protein Secretion from Rat Sertoli Cells in Primary Culture, *Endocrinology 113*, 2284, 1983 (*379*)
	Rat, SD, female, 26 days	Luteal	Deoxyribonuclease I: 0.0004%	Medium 199	Azhar, S. and Reaven, E.: Effect of Antimicrotubule Agents on Microtubules and Steroidogenesis in Luteal Cells, *Am J Physiol 243*, E380, 1982 (*290*)
	Rat, SD, male	Leydig	Collagenase: 0.1%	Krebs Ringer bicarbonate buffer	Ramachandran, J., and Sairam, M.R.: The Effects Of Interstitial Cell-Stimulating Hormone, Its Subunits,and Recombinants on Isolated Rat Leydig Cells, *Arch Biochem Biophys 167*, 294, 1975 (*303*)

Scales

Scales

Species		Cell(s)	Enzyme(s)	Medium	Reference
Fish	Goldfish (*Carassius Auratus L.*)	Pigment, xanthopores	Deoxyribonuclease I: 0.005%	Medium 199 w/BSA	Lo, S., Grabowski, S., Lynch, T., Kern, D., Taylor, J.T., and Chen, T.: Isolation of xanthophores from the goldfish, *In Vitro 18*, 356, 1982 (*518*)

Skin

Skin

Species		Cell(s)	Enzyme(s)	Medium	Reference
Frog	Frog	Epidermal	Trypsin: 0.18%	Barth's solution, CMF	Nishikawa, A., Shimizu-Nishikawa, K., and Miler, L.: Isolation, characterization, and in vitro culture of larval and adult epidermal cells of the frog Xenopus laevis, *In Vitro Cell Dev Biol 26*, 1128, 1990 (*1287*)
Human	Human, 18-50 years	Sweat duct	Collagenase: 0.03%	MEM	Bell, C. and Quinton, P.: Effects of Media Buffer Systems on Growth and Electrophysiologic Characteristics of Cultured Sweat Duct Cells, *In Vitro Cell Dev Biol 27*, 47, 1991 (*466*)
	Human	Fibroblasts	Trypsin: 0.25%	CMF solution	Limat, A., Hunziker, T., Boillat, C., Noser, F., and Wiesmann, U.: Postmitotic Human Dermal Fibroblasts Preserve Intact Feeder Properties for Epithelial Cell Growth After Long-Term Cryopreservation, *In Vitro Cell Dev Biol 26*, 709, 1990 (*447*)
	Human	Melanocytes, skin/ foreskin	Trypsin: 0.25%	PBS	Peacocke, M., Yaar, M., Mansur, C., Chao, M., and Gilchrest, B.: Induction of Nerve Growth Factor Receptors on Cultured Human Melanocytes, *Proc Natl Acad Sci U S A 85*, 5282, 1988 (*660*)
	Human	Keratinocytes	Trypsin: 0.25%	DMEM	Dover, R. and Potten, C.: Cell Cycle Kinetics of Cultured Human Epidermal Keratinocytes, *J Invest Dermatol 80*, 423, 1983 (*683*)
	Human, fetal	Smooth muscle, fibroblasts	Trypsin: 0.055%	DMEM	Davies, P. and Kerr, C.: Modification of LDL Metabolism by Growth Factors in Cultured Vascular Cells and Human Skin Fibroblasts, *Biochim Biophys Acta 712*, 26, 1982 (*322*)
	Human, 18-30 years, male, female	Fibroblasts	Trypsin: 0.1%	HBSS	McCoy, B., Galdun, J., and Cohen, I.: Effects of Density and Cellular Aging On Collagen Synthesis and Growth Kinetics in Keloid and Normal Skin Fibroblasts, *In Vitro 18 (1)*, 79, 1982 (*1129*)

Spleen

Spleen

Species		Cell(s)	Enzyme(s)	Medium	Reference
Mouse	Mouse	Spleen, bone marrow endothelial	Collagenase Type 4: 0.3-1.0% Deoxyribonuclease I: 20 u/ml	PBS	Shi, C., Jia, T., Mendez-Ferrer, S., Hohl, T., Serbina, N., Lipuma, L., Leiner, I., Li, M., Frenette, P. and Pamer, E.: Bone Marrow Mesenchymal Stem and Progenitor Cells Induce Monocyte Emigration in Response to Circulating Toll-Like Receptor Ligands., *Immunity 34*, 590, 2011 (*10641*)
	Mouse	Dendritic	Collagenase: 300 u/ml Deoxyribonuclease I: 0.002%	RPMI 1640	Abe, K., Nguyen, K., Fine, S., Mo, J., Shen, C., Shenouda, S., Corr, M., Jung, S., Lee, J., Eckmann, L. and Raz, E.: Conventional Dendritic Cells Regulate the Outcome of Colonic Inflammation Independently of T Cells., *Proc Natl Acad Sci U S A 104*, 17022, 2007 (*10356*)

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Stem

Species		Cell(s)	Enzyme(s)	Medium	Reference
Human	Human	Dental pulp derived stem cell	Collagenase Type 1: 0.3% Neutral Protease: 0.4%	DMEM	Sakai, K., Yamamoto, A., Matsubara, K., Nakamura, S., Naruse, M., Yamagata, M., Sakamoto, K., Tauchi, R., Wakao, N., Imagama, S., Hibi, H., Kadomatsu, K., Ishiguro, N. and Ueda, M: Human Dental Pulp-Derived Stem Cells Promote Locomotor Recovery After Complete Transection of the Rat Spinal Cord by Multiple Neuro-Regenerative Mechanisms. ,*J Clin Invest 122*, 80, 2012
	Human	Umbilical cord stromal stem	Collagenase Type 4: 0.08% Neutral Protease: 0.138% Hyaluronidase: 0.02%	DMEM	Farias, V., Linares-Fernandez, J., Penalver, J., Paya Colmenero, J., Ferron, G., Duran, E., Fernandez, R., Olivares, E., O'Valle, F., Puertas, A., Oliver, F. and Ruiz de Almodovar, J.: Human Umbilical Cord Stromal Stem Cell Express CD10 and Exert Contractile Properties., *Placenta 32*, 86, 2011 (*10683*)
	Human, male	Adipose derived adult stem cells	Collagenase Type 1: 0.1%	DMEM	Lei, L., Liao, W., Sheng, P., Fu, M., He, A. and Huang, G.: Biological Character of Human Adipose-Derived Adult Stem Cells and Influence of Donor Age on Cell Replication in Culture., *Sci China C Life Sci Vol. 50*, 320, 2007 (*10517*)
	Human	Pancreatic cancer stem cells	Collagenase Type 4: 200 u/ml	Medium 199	Li, C., Heidt, D., Dalerba, P., Burant, C., Zhang, L., Adsay, V., Wicha, M., Clarke, M. and Simeone, D.: Identification of Pancreatic Cancer Stem Cells., *Cancer Res 67*, 1030, 2007 (*10514*)
	Human, 8-12 year	Tendon stem/progenitor	Collagenase Type 1: 0.3% Neutral Protease: 0.4%	DMEM	Bi Yanming, Ehirchiou Driss, Kilts Tina M, Inkson Colette A, Embree Mildred C, Sonoyama Wataru, Li Li, Leet Arabella I, Seo Byoung-Moo, Zhang Li, Shi Songtao, Young Marian F: Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche, *Nat Med 13*, 1219-27, 2007 (*10337*)
	Human	Embryoinic system	Neutral Protease: 1% Collagenase Type 4: 0.1%	DMEM	Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM: Embryonic stem cell lines derived from human blastocysts, *Science 282*, 1145-7, 1998 (*10318*)
	Human, 8-12 year	Tendon stem/progenitor	Collagenase Type 1: 0.3% Neutral Protease: 0.4%	DMEM	Bi Yanming, Ehirchiou Driss, Kilts Tina M, Inkson Colette A, Embree Mildred C, Sonoyama Wataru, Li Li, Leet Arabella I, Seo Byoung-Moo, Zhang Li, Shi Songtao, Young Marian F: Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche, *Nat Med 13*, 1219-27, 2007 (*10337*)
	Human, adlut	Human skin mast cells	Collagenase Type 2: 0.15% Hyaluronidase: 0.07% Deoxyribonuclease I: 0.03%	HBSS	Kambe, N, Kambe, M, Kochan, J, and Schwartz ,L.: Human Skin-derived Mast Cells Can Proliferate While Retaining Their Characteristic Functional and Protease Phenotypes, *Blood 97*, 2045, 2001 (*9803*)
	Human	Mesenchymal stem	Collagenase Type 1: 0.075%	DMEM	Kern Susanne, Eichler Hermann, Stoeve Johannes, Kluter Harald, Bieback Karen: Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue, *Stem Cells 24*, 1294-301, 2006 (*10329*)
	Human, 40-65 year	Adult human adipose stem cells	Collagenase Type 2: 0.075%	DMEM	Sun, N., Panetta, N., Gupta, D., Wilson, K., Lee, A., Jia, F., Hu, S., Cherry, A., Robbins, R., Longaker, M. and Wu, J.: Feeder-Free Derivation of Induced Pluripotent Stem Cells from Adult Human Adipose Stem Cells., *Proc Natl Acad Sci U S A 106*, 15720, 2009 (*10525*)
	Human	Umbilical cord stromal stem	Collagenase Type 4: 0.08% Neutral Protease: 0.138% Hyaluronidase: 0.02%	DMEM	Farias, V., Linares-Fernandez, J., Penalver, J., Paya Colmenero, J., Ferron, G., Duran, E., Fernandez, R., Olivares, E., O'Valle, F., Puertas, A., Oliver, F. and Ruiz de Almodovar, J.: Human Umbilical Cord Stromal Stem Cell Express CD10 and Exert Contractile Properties., *Placenta 32*, 86, 2011 (*10683*)
Monkey	Monkey	Embryonic stem	Collagenase Type 4: 0.08%	DMEM	Chen, S., Revoltella, R., Papini, S., Michelini, M., Fitzgerald, W., Zimmerberg, J.,and Margolis, L.: Multilineage Differentiation of Rhesus Monkey Embryonic Stem cells in Three-dimensional Culture Systems, *Stem Cells 21(3)*, 281, 2003 (*9805*)
Mouse	Mouse, 8-10 week	Adipose derived stem	Collagenase Type 1: 0.025%	HBSS	Sugii, S., Kida, Y., Berggren, W. and Evans, R.: Feeder-Dependent and Feeder-Independent iPS Cell Derivation from Human and Mouse Adipose Stem Cells., *Nat Protoc 6*, 346, 2011 (*10493*)
	Mouse	Spleen, bone marrow endothelial	Collagenase Type 4: 0.3-1.0% Deoxyribonuclease I: 20 u/ml	PBS	Shi, C., Jia, T., Mendez-Ferrer, S., Hohl, T., Serbina, N., Lipuma, L., Leiner, I., Li, M., Frenette, P. and Pamer, E.: Bone Marrow Mesenchymal Stem and Progenitor Cells Induce Monocyte Emigration in Response to Circulating Toll-Like Receptor Ligands., *Immunity 34*, 590, 2011 (*10641*)
	Mouse	Neural progenitor cell	Papain: 10 ul/ml	PBS	Hutton, S. and Pevny, L.: Isolation, Culture, and Differentiation of Progenitor Cells from the Central Nervous System., *Cold Spring Harb. Protoc. 11*, 5077, 2008 (*10532*)
	Mouse	Liver epithelial progenitor cells	Collagenase Type 4: 0.1% Deoxyribonuclease I: 0.05%	DMEM	Li Wen-Lin, Su Juan, Yao Yu-Cheng, Tao Xin-Rong, Yan Yong-Bi, Yu Hong-Yu, Wang Xin-Min, Li Jian-Xiu, Yang Yong-Ji, Lau Joseph T Y, Hu Yi-Ping: Isolation and characterization of bipotent liver progenitor cells from adult mouse, *Stem Cells 24*, 322-32, 2006 (*10248*)
Rat	Rat, SD, newborn	Neutral stem cells, Schwann cells	Trypsin: 0.25% Collagenase: 0.16%	DMEM/F12	Zeng Yuan-Shan, Ding Ying, Wu Li-Zhi, Guo Jia-Song, Li Hai-Biao, Wong Wai-Man, Wu Wu-Tian: Co-transplantation of schwann cells promotes the survival and differentiation of neural stem cells transplanted into the injured spinal cord, *Dev Neurosci 27*, 20-6, 2005 (*10109*)
	Rat, male, 200-250 g	Neural stem cells	Papain: 0.09% Deoxyribonuclease I: 0.1%	EBSS	Gobbel GT, Choi SJ, Beier S, Niranjan A: Long-term cultivation of multipotential neural stem cells from adult rat subependyma, *Brain Res 980*, 221, 2003 (*10051*)

Thymus

Species		Cell(s)	Enzyme(s)	Medium	Reference
Mouse	Mouse BALB/c, C3H, C57BL/6, 1-28 day	Epithelial, thymus	Neutral Protease: 1.5 µg/ml	DMEM	Ropke, C., van Deurs, B., and Petersen, O.: Short-term Cultivation of Murine Thymic Epithelial Cells in a Serum-Free Medium, *In Vitro Cell Dev Biol 26*, 671, 1990 (*1288*)
	Mouse, Swiss, 6 wk	Thymus	Collagenase Type 3: 150 u/ml	DMEM	Jones, K. and Pierre, R.: Analysis of Cellular Heterogeneity in Mouse Thymus Cultures, *In Vitro 17*, 431, 1981 (*511*)
Rat	Rat ACI/NMs X BUF/Mna F1, male, 28 months Rat, ACI/MNs male, 8 weeks (also bovine, adult)	Epthelial	Collagenase Type 3: 0.1%	Eagle's MEM Serum-free	Masuda, A., Ohtsuka, K., and Matsuyama, M.: Establishment of Functional Epithelial Cell Lines from a Rat Thyoma and a Rat Thymus, *In Vitro Cell Dev Biol 26*, 713, 1990 (*448*)

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Thyroid/Parathyroid

Thyroid/Parathyroid

Species		Cell(s)	Enzyme(s)	Medium	Reference
Bovine	Bovine, adult	Parathyroid	Deoxyribonuclease I: 0.005%,	HEPES Ham's F10	Nygren, P., Gylfe, E., Larsson, R., Johansson, H., Juhlin, C., Klareskoq, L., Akerstrom, G., and Rastad, J.: Modulation of the Ca2+-Sensing Function of Parathyroid Cells *In Vitro* and in Hyperparathyroidism, *Biochim Biophys Acta 968*, 253, 1988 (*337*)
	Bovine	Parathyroid glands	Deoxyribonuclease I: 0.0075%	HEPES buffer	Wallace, J., and Scarpa, A.: Regulation of Parathyroid Hormone Secretion *in Vitro* by Divalent Cations, *J Biol Chem 257*, 10613, 1982 (*555*)
	Bovine	Parathyroid	Deoxyribonuclease I: 0.004%	Eagle's #2 medium without bicarbonate	Brown, E., Hurwitz, S., and Aurbach, G.: Preparation of Viable Isolated Bovine Parathyroid Cells, *Endocrinology 99*, 1582, 1976 (*385*)
	Bovine (also porcine)	Thyroid	Trypsin: 0.004%	EBSS	Tong, W.: The Isolation and Culture of Thyroid Cells, *Meth Enzymol 32*, 745, 1974 (*636*)
Chicken	Chick embryo	Thyroid Muscle Heart	Collagenase: 0.25%	Tyrode's saline, potassium free	Hilfer, S., and Brown, J.: Collagenase. Its Effectiveness as a Dispersing Agent for Embryonic Chick Thyroid and Heart, *Exp Cell Res 65*, 246, 1971 (*401*)
	Chicken, Rhode Island Red, embryo	Thyroid follicular	Collagenase: 0.2%	Tyrode's solution, CMF	Spooner, B.: The Expression of Differentiation by Chick Embryo Throid in Cell Culture. I. Functional and Fine Structural Stability in Mass and Clonal Culture, *J Cell Physiol 75*, 33, 1970 (*682*)
Ovine	Sheep	Thyroid	Collagenase: 0.2%	Puck's Saline F	Kerkof, P.: Preparation of Primary Cultures of Ovine Thyroid Gland Cells, *J Tiss Cul Meth 7*, 23, 1982 (*1289*)
Porcine	Porcine (also bovine)	Thyroid	Trypsin: 0.004%	EBSS	Tong, W.: The Isolation and Culture of Thyroid Cells, *Meth Enzymol 32*, 745, 1974 (*636*)

Tonsil

Tonsil

Species		Cell(s)	Enzyme(s)	Medium	Reference
Human	Human	Tonsillar mononuclear cells	Collagenase Type 1: 210 u/ml Deoxyribonuclease I: 90 u/ml	RPMI	Grammer AC, McFarland RD, Heaney J, Darnell BF, Lipsky PE: Expression, regulation, and function of B cell-expressed CD154 in germinal centers, *J Immunol 163*, 4150-9, 1999 (*10230*)

Tumor

Tumor

Species		Cell(s)	Enzyme(s)	Medium	Reference
Hamster	Hamster, 6 week old	Buccal pouch	Neutral Protease: 0.24%	CMF HBSS	Min, B., Kim, K., Cherrick, H., and Park, N.: Three Cell Lines from Hamster Buccal Pouch Tumors Induced by Topical 7,12-Dimethylbenz(a)Anthracene, Alone or in Conjunction with Herpes Simplex Virus Inoculation, *In Vitro Cell Dev Biol 27A*, 128, 1991 (*457*)
Human	Human	Epithelial	Collagenase: 2.0%	DMEM/Ham's F-12	Emerman, J. and Wilkinson, D.: Routine Culturing of Normal, Dysplastic and Malignant Human Mammary Epithelial Cells from Small Tissue Samples, *In Vitro Cell Dev Biol 26*, 1186, 1990 (*429*)
	Human	Tumor	Neutral Protease: 0.24%	DMEM/Ham's F-12	Boyd, J., Rinehart Jr., C., Walton, L., Siegal, G. and Kaufman, D.: Ultrastructural Characterization of Two New Human Endometrial Carcinoma Cell Lines and Normal Human Endometrial Epithelial Cells Cultured on Extracellular Matrix, *In Vitro Cell Dev Biol 26*, 701, 1990 (*446*)
	Human	Epthelial and tumor Colon	Collagenase: 300 u/ml	PBS medium 199 or medium F 12	Friedman, E., Higgins, P., Lipkin, M., Shinya, H., and Gelb, A.: Tissue Culture of Human Epithelial Cells from Benign Colonic Tumors, *In Vitro 17*, 632, 1981 (*514*)
	Human	Tumor, breast	Neuraminidase: 0.8 u/ml	HBSS	Leung, C., and Shiu, R.: Morphological and Proliferative Characteristics of Human Breast Tumor Cells Cultured on Plastic and in Collagen Matrix, *In Vitro 18*, 476, 1981 (*521*)
	Human	Melanoma Metastatic tumors	Collagenase Type 3: 0.10%	DMEM	Creasey, A., Smith, H., Hackett, A., Fukuyama, K., Epstein, W., and Madin, S.: Biological Properties of Human Melanoma Cells in Culture, *In Vitro 15*, 342, 1979 (*503*)
	Human	Mammary tumors, hard	Collagenase: 0.10%	RPMI-1640 w/ 5% Fetal Calf Serum	Lasfargues, E.: ,*Tissue Culture Methods/Applications*,Kruse, P., and Patterson, M., Academic Press, 45, 1973 (*1293*)
Mouse	Mouse BALB/cfC3H	Mammary tumors Epithelial	Collagenase: 1.0%	HBSS	Yang, J., Guzman, R., Richards, J., and Nandi, S.: Primary Cultures of Mouse Mammary Tumor Epithelial Cells Embedded in Collagen Gels, *In Vitro 16*, 502, 1980 (*507*)
Rat	Rat ACI/NMs X BUF/Mna F1, male, 28 months, rat, ACI/MNs male, 8 weeks	Epthelial	Collagenase Type 3: 0.1%	Eagle's MEM Serum-free	Masuda, A., Ohtsuka, K., and Matsuyama, M.: Establishment of Functional Epithelial Cell Lines from a Rat Thyoma and a Rat Thymus, *In Vitro Cell Dev Biol 26*, 713, 1990 (*448*)
	Rat	Yolk sac tumor	Trypsin: 0.01%	DMEM	Brennan, M., Oldberg, A., Hayman, E., and Ruoslahti, E.: Effect of a Proteoglycan Produced by Rat Tumor Cells on Their Adhesion to Fibronectin-Collagen Substrata, *Cancer Res 43*, 4302, 1983 (*353*)
	Rat	Ascites hepatoma	Trypsin: 0.1%	Phosphate buffer (see reference)	Essner, E.: Experiments on an Ascites Hepatoma. I. Enzymatic Digestion and Alkaline Degradation of the Cementing Substance and Separation of Cells, in Tumor Islands, *Exp Cell Res 7*, 430, 1954 (*403*)

[CF=Calcium Free • CMF=Calcium Magnesium Free • BSS=Balanced Salt Solution]

Worthington Collagenase Sampling Program

The lot-to-lot variation which is typical of partially purified enzyme preparations such as Worthington collagenase Types 1-4 and Animal Origin Free CLSAF types makes it important to pre-test a particular lot of enzyme you are planning to use in your experiment. Many years ago we found that the most practical approach for the researcher is to presample several different lots of collagenase at a time and select the best of the group. As the world's leading manufacturer of collagenase, Worthington is able to offer the greatest number of different lots at any given time and recommend specific lots for an application.

There is no charge for participating in the collagenase sampling program. Under the program, individual researchers are provided with 100 mg samples of up to three different lots of collagenase for evaluation in their own assay systems. A period of 60 days is allowed for your evaluation of these samples. A minimum of 3 grams of each lot will be placed on HOLD, reserved in your name. When you determine which lot performs best for you, simply specify the lot desired when ordering. **The only requirement, once a suitable lot of collagenase is found, is that you purchase a minimum of 3 grams of the material.**

To become part of this program, or to discuss any of the Worthington products, just call our **Technical Service group toll-free at 800.445.9603** from anywhere in the United States or Canada or e-mail **techservice@worthington-biochem.com**

International customers should check our International Distributor listing for a distributor. If you do not have a Worthington Distributor for your country, please contact International Sales or Technical Service.

Related Worthington Products

The enzymes included in this kit are all samples of standard Worthington products; however, some of the package sizes used in the kit are non-standard. The following pages list the standard package sizes for each of the products along with brief descriptions and catalog numbers. Worthington also offers multiple grades of most of these same enzymes. Refer to the Catalog and Price List for a complete listing. To obtain a copy call Customer Service at:

800.445.9603.

Please note that bulk purchases are also possible with each of the products. If your experiments require substantial quantities of one or more of these enzymes, we recommend that you contact our Customer Service department to discuss bulk pricing.

Ordering information along with our discount structure appears inside the back cover of this guide. If you have any questions concerning our products, please do not hesitate to call Technical Service toll-free at 800.445.9603 from anywhere in the United States or Canada or at 732.942.1660 from elsewhere. Twenty-four hour FAX service is available at 732.942.9270

Cell Isolation Product List

Product		Catalog #	Size	Code
Cell Isolation Optimizing System				**CIT**
A complete method development kit containing an assortment of enzymes most frequently used in tissue dissociation and cell isolation procedures. Includes instructions, references and strategies for the handling, use and optimization of enzymatic cell isolation methods to achieve maximum yield of viable cells. Kit includes 500 mg of each of four types of collagenase, 500 mg trypsin, 50 ku hyaluronidase, 100 mg elastase, 100 mg papain, 25 mg DNase I, 10 mg neutral protease (Dispase) and 100 mg trypsin inhibitor. Store at 2-8°C.		LK003200	1 bx	
Chymotrypsin, Alpha, 1X				**CDAG**
1X crystallized as zymogen and activated. Dialyzed against 1mM HCl and lyophilized. Store at 2-8°C.	≥35 units per mg protein	LS001333 LS001334 LS001332	1 gm 10 gm Bulk	
Chymotrypsin, Alpha, 3X				**CDI**
3X crystallized alpha chymotrypsin which is an activation product of a 3X crystallized zymogen. Dialyzed against 1mM HCl and lyophilized. Store at 2-8°C.	≥45 units per mg protein	LS001448 LS001450 LS001451 LS001453	250 mg 1 gm 10 gm Bulk	
Chymotrypsin, Alpha, Purified				**CDS**
Chromatographically prepared by the procedure of Yapel, *et al., J. Amer. Chem. Soc., 88,* 2573 (1966). A lyophilized powder. Store at 2-8°C.	≥45 units per mg protein	LS001475 LS001479 LS001477	100 mg 1 gm Bulk	
Chymotrypsin, Alpha, TLCK Treated				**CDTLCK**
3X crystallized and treated with 1-chloro-3-tosylamido-7-amino-2-heptanone (TLCK) to inhibit trypsin activity [Shaw, *et al., Biochemistry, 4*, 2219 (1965)]. Dialyzed against 1 mM HCl to remove autolysis products and low molecular weight contaminants. Supplied as a dialyzed, lyophilized powder. Store at 2-8°C.	≥45 units per mg protein	LS001430 LS001432 LS001434 LS001438	25 mg 100 mg 1 gm Bulk	
Collagen				**CL**
Type I collagen prepared by the method of Einbinder and Schubert, *J. Biol. Chem., 188,* 335 (1951). Supplied as a shredded, lyophilized, insoluble preparation. Store at 2-8°C.		LS001654 LS001652 LS001656 LS001658	1 gm 5 gm 10 gm Bulk	
Collagen, Soluble				**CLCS**
Type I collagen supplied as a 6mg/ml liquid preparation in 75mM sodiuim citrate, pH 3.6 - 4.0, containing 0.01% merthiolate as a preservative. Store at 2-8°C REQUIRES SPECIAL SHIPPING: ICE PACK. Note: Contains thimerisol as a preservative; proper disposal required.	Less than 20 minutes gel time	LS001663	Bulk	

Cell Isolation Product List

Product		Catalog #	Size	Code
Collagenase, Animal Origin-Free				**CLSAFA**
Collagenase derived from cultures grown in animal-free medium. Suitable for applications needing to avoid introduction of anumal derived pathogens into bioprocessing procedures. Store at 2-8°C.	≥150 units per mg dry weight	LS004152 LS004154 LS004156 LS004158	100 mg 1 gm 5 gm Bulk	
Collagenase, Type 1				**CLS-1**
The original balance of enzymatic activities. Each lot assayed for collagenase, caseinase, clostripain and tryptic activities. Suggested for epithelial, liver, lung and adrenal primary cell isolations. A dialyzed, lyophilized powder. Store at 2-8°C.	≥125 units per mg dry weight	LS004194 LS004196 LS004197 LS004200	100 mg 1 gm 5 gm Bulk	
Collagenase, Type 2				**CLS-2**
Prepared to contain higher clostripain activity. Suggested for bone, heart, liver, thyroid and salivary primary cell isolation. Supplied as a dialyzed, lyophilized powder. Store at 2-8°C.	≥125 units per mg dry weight	LS004174 LS004176 LS004177 LS004179	100 mg 1 gm 5 gm Bulk	
Collagenase, Type 3				**CLS-3**
Lower in secondary proteolytic contaminant activities but wIth typical collagenase activity. Suggested for mammary primary cell isolation. A dialyzed, lyophilized powder. Store at 2-8°C.	≥100 units per mg dry weight	LS004180 LS004182 LS004183 LS004185	100 mg 1 gm 5 gm Bulk	
Collagenase, Type 4				**CLS-4**
Prepared to contain lower tryptic activity levels to limit damage to membrane proteins and receptors but with normal to above normal collagenase activity. Suggested for pancreatic islet primary isolation. A dialyzed, lyophilized powder. Store at 2-8°C.	≥160 units per mg dry weight	LS004186 LS004188 LS004189 LS004191	100 mg 1 gm 5 gm Bulk	
Collagenase, Purified				**CLSPA**
Chromatographically purified. ≤50 caseinase units per milligram. Supplied as a lyophilized powder. Store at 2-8°C.	≥500 units per mg dry weight	LS005275 LS005273 LS005277	4 ku 10 ku Bulk	
Collagenase, Type 1, Filtered				**CLSS-1**
Collagenase, Type 1 (Code: CLS-1), which is filtered through a 0.22 micron membrane and lyophilized in vials to contain > 50 milligrams or 1 gram per vial. Store at 2-8°C.	≥125 units per mg dry weight	LS004214 LS004216 LS004217	50 mg 5x50 mg 1 gm	

Cell Isolation Product List

Product		Catalog #	Size	Code
Collagenase, Type 2, Filtered				**CLSS-2**
Collagenase, Type 2 (Code: CLS-2), which is filtered through a 0.22 micron membrane and lyophilized in vials to contain > 50 milligrams or 1 gram per vial. Store at 2-8°C.	≥125 units per mg dry weight	LS004202 LS004204 LS004205	50 mg 5x50 mg 1 gm	
Collagenase, Type 3, Filtered				**CLSS-3**
Collagenase, Type 3 (Code: CLS-3), which is filtered through a 0.22 micron membrane and lyophilized in vials to contain ≥ 50 milligrams per vial. Store at 2-8°C.	≥100 units per mg dry weight	LS004206 LS004208	50 mg 5x50 mg	
Collagenase, Type 4, Filtered				**CLSS-4**
Collagenase, Type 4 (Code: CLS-4), which is filtered through a 0.22 micron membrane and lyophilized in vials to contain > 50 milligrams or 1 gram per vial. Store at 2-8°C.	≥160 units per mg dry weight	LS004210 LS004212 LS004209	50 mg 5x50 mg 1 gm	
Deoxyribonuclease I, Ribonuclease & Protease Free, Solution				**DPRFS**
Molecular Biology Grade. Chromatographically purified to remove RNase and protease. Supplied as a solution at approximately 2 Kunitz units per microliter (approximately 1 mg/ml) containing 50% glycerol and 1mM calcium chloride. Store at 2-8°C or -20°C.	≥2,000 Kunitz units per ml	LS006342 LS006344 LS006348	100 un 500 un Bulk	
Deoxyribonuclease I, Ribonuclease & Protease Free				**DPRF**
Molecular Biology Grade. Chromatographically purified to remove RNase and protease. Lyophilized in vials. Each 10,000 unit vial contains 2 mg glycine, 2µmoles calcium, and ≥10,000 units of DNase I. Each 2,500 unit vial contains 0.5mg glycine, 0.5µmoles calcium, and ≥2,500 units of DNase I. Dissolving the entire 10,000 unit vial in 5 ml, or the entire 2,500 unit vial in 1.25 ml, provides the equivalent of a 1 mg/ml solution. (ku = 1000un) Store at 2-8°C. PROTECT FROM MOISTURE	≥2,000 units per mg dry weight	LS006331 LS006333 LS006334	2500 un 10 ku Bulk	
Deoxyribonuclease I				**DPFF**
Chromatographically purified. A lyophilized powder containing glycine as a stabilizer. Contains ≤ 0.0005% RNase. Store at 2-8°C. PROTECT FROM MOISTURE. RNase & Protease Free	≥2,000 Kunitz units per mg dry weight	LS006330 LS006328 LS006332	25 ku 125 ku Bulk	
Deoxyribonuclease I				**D**
Chromatographically purified. A lyophilized powder with glycine as a stabilizer. Store at 2-8°C. PROTECT FROM MOISTURE	≥2,000 Kunitz units per mg dry weight	LS002004 LS002006 LS002007 LS002009	5 mg 20 mg 100 mg Bulk	

Cell Isolation Product List

Product		Catalog #	Size	Code
Deoxyribonuclease I, Filtered				**DCLS**
Filtered through a 0.22 micron membrane and lyophilized in vials. Material is not tested for pyrogenicity. Store at 2-8°C. PROTECT FROM MOISTURE	≥2,000 units per mg dry weight	LS002058 LS002060	11 mg 25 mg	
Deoxyribonuclease I, Standard Vial				**DSV**
Lyophilized in vials for assay standardization. Labeled to show established activity. Not suitable for assays at neutral pH. Store at 2-8°C. PROTECT FROM MOISTURE	~2,000 Kunitz units per vial	LS002173 LS002172	2 ku 5x2 ku	
Deoxyribonuclease I				**DP**
Partially purified. A lyophilized powder. Store at 2-8°C. PROTECT FROM MOISTURE	≥2,000 Kunitz units per mg dry weight	LS002138 LS002139 LS002140 LS002141	25 mg 100 mg 1 gm Bulk	
Deoxyribonuclease I				**DPB**
Partially purified. Supplied as lyophilized powder. Store at 2-8°C. PROTECT FROM MOISTURE	≥1,250 Kunitz units per mg dry weight	LS002145 LS002147 LS002149	100 mg 1 gm Bulk	
Deoxyribonuclease I, Recombinant				**DR1**
Recombinant protein produced in *Pichia pastoris*. Free of animal derived components, RNases and proteases. Chromatographically purified. A lyophilized powder containing glycine as a stabilizer. Store at 2-8°C. PROTECT FROM MOISTURE	≥5,000 units per mg protein	LS006361 LS006362 LS006360	10 ku 50 ku Bulk	
Deoxyribonuclease I, Recombinant Solution				**DR1S**
Recombinant protein produced in *Pichia pastoris*. Free of animal derived components, RNases and proteases. Chromatographically purified. A liquid preparation in 5mM Calcium Acetate, 4mg/ml glycine, pH 5.0 and 50% glycerol. Supplied with 10x reaction buffer. Store at -20°C. REQUIRES SPECIAL SHIPPING: ICE PACK	≥2 units per microliter	LS006353 LS006355 LS006357	2 ku 5 x 2 ku Bulk	

Cell Isolation Product List

Product		Catalog #	Size	Code
Elastase, Suspension				**ES**
2X crystallized. Supplied as an aqueous suspension. This preparation must be diluted to dissolve the enzyme. The diluted enzyme should be 0.22 micron filtered before use. Suitable for the isolation of Type II lung cells. Store at 2-8°C. DO NOT FREEZE REQUIRES SPECIAL SHIPPING: ICE PACK.	≥3 units per mg protein	LS002274 LS002279 LS002280 LS002276	25 mg 100 mg 1 gm Bulk	
Elastase, Purified				**ESFF**
Chromatographically purified. A lyophilized powder. Store at 2-8°C. REQUIRES SPECIAL SHIPPING: ICE PACK	≥8 units per mg protein	LS006363 LS006365 LS006367	5 mg 20 mg Bulk	
Elastase, Lyophilized				**ESL**
2X Crystallized, (Code: ESL), supplied as a dialyzed, lyophilized powder. The enzyme should be 0.22 micron filtered after reconstitution and prior to use. Suitable for the isolation of Type II lung cells. Store at 2-8°C.	≥3 units per mg protein	LS002290 LS002292 LS002294 LS002298	25 mg 100 mg 1 gm Bulk	
Hepatocyte Isolation System				**HIS**
The package contains sufficient materials for five separate adult rat liver perfusions including five single use CLSH enzyme vials, Five single use DNase vials, 10X CMF-Hank's Balanced Salt Solution, L-15 Media Powder, 0.15M MOPS buffer. 7.5% Sodium Bicarbonate Solution and optimized protocol. Store at 2-8°C.		LK002060	bx	
HIS-Collagenase/Elastase				**CLSH**
Worthington collagenase (Code: CLS-1) and elastase (Code: ESL), filtered through 0.22µm pore size membrane, and lyophilized. Before use, reconstitute with the L-15/MOPS solution and swirl gently to dissolve contents as directed in the following procedure. Store unreconstituted vials at 2–8°C.		LK002066 LK002067	1 Vl 5 Vl	
HIS Kit, DNase Vial				**D2**
A component of the Hepatocyte Isolation kit containing 1,000 Units DNase I each, 5 Vials Worthington DNase I (Code: D), filtered through 0.22µm pore size membrane, and lyophilized. Before use, reconstitute with L-15/MOPS solution and swirl gently to dissolve contents as directed in the procedure. Store unreconstituted vials at 2–8°C.	≥1,000 units per vial	LK003170 LK003172	1 vi 5 vi	
Hank's Balanced Salt Solution (HBSS-CMF) 10X Solution				**HBSS10**
10X CMF-HBSS Concentrate, 1 bottle, 500ml Sterile calcium- and magnesium-free Hank's Balanced Salt Solution (CMF-HBSS). The solution is used for washing and perfusing the liver prior to the addition of the dissociating enzyme solution.		LK002064	1 ea	

Cell Isolation Product List

Product		Catalog #	Size	Code
L-15 Media Powder				**L15NK**
Leibovitz L-15 media powder, a component of the HIS kit. Reconstitute entire contents of pouch, QS to 1 liter with cell culture grade water, and 0.22 micron filter. Suitable for cell isolation and culture applications. Store at 2-8°C.		LK003250	1 ea	
0.15M, MOPS Buffer, HIS				**MOPS**
0.15M MOPS, pH 7.5, 0.22u filtered. Buffer concentrate used to buffer the constituted Leibovitz L-15 media in Hepatocyte Isolation System. Store at 2-8°C.		LK002070	1 ea	
Sodium Bicarbonate 7.5%				**NAH**
7.5% Sodium Bicarbonate ($NaHCO_3$),1 bottle, 100ml 7.5% Sodium bicarbonate concentrate, used to buffer the diluted CMF-HBSS. Store at 2-8°C.		LK002069	1 ea	
Hyaluronidase				**HSE**
A partially purified, dialyzed, lyophilized powder. Store at -20°C.	≥300 USP/NF units per mg dry weight	LS002594 LS002592 LS002591	50 ku 300 ku Bulk	
Hyaluronidase, Purified				**HSEP**
Chromatographically purified. A dialyzed, lyophilized powder. Store at -20°C.	≥3,000 USP/NF units per mg dry weight	LS005477 LS005475 LS005474 LS005479	5 ku 15 ku 30 ku Bulk	
Lysozyme				**LY**
A lyophilized powder containing sodium chloride and acetate. Store at 2-8°C.	≥5,000 units per mg dry weight	LS002880 LS002881 LS002883	1 gm 10 gm Bulk	
Lysozyme, Purified, Salt Free				**LYSF**
A dialyzed, lyophilized powder. Store at 2-8°C.	≥8,000 units per mg dry weight	LS002931 LS002933 LS002934	1 gm 5 gm Bulk	
Neonatal Cardiomyocyte Isolation System				**NCIS**
Kit for performing five separate tissue dissociations each containing up to twelve hearts. Contains single use vials of purified collagenase and trypsin, CMF-HBSS, Leibovitz L-15 media and Falcon cell strainers along with a detailed protocol. The kit is use-tested by Worthington to assure performance. Store at 2-8°C.		LK003300 LK003303	1 ki 3 ki	

Cell Isolation Product List

Product		Catalog #	Size	Code
Collagenase Vial, NCIS				**CLSPANK**
A component of the NCIS kit. This material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 5 ml of HBSS or equivalent yields a solution of 300 units/ml of collagenase, Code: CLSPA. Suitable for cell isolation and culture applications. Store at 2-8°C.	≥500 units per mg dry weight	LK003240 LK003245	1 vi 5 vi	
Trypsin Vial, NCIS				**TRLSNK**
A component of the NCIS kit. This material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 2 ml of HBSS yields a solution of 500µg/ml of trypsin, Code: TRLS. Suitable for cell isolation and culture applications. Store at 2-8°C.	≥180 units per vial	LK003220 LK003225	1 vi 5 vi	
Inhibitor Vial, NCIS				**SICNK**
A component of the NCIS kit. This material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 1 ml of HBSS or equivalent yields a solution of 2 mg/ml of trypsin inhibitor, Code: SIC. Suitable for cell isolation and culture applications. Store at 2-8°C.	1 mg inhibits at least 0.75 mg trypsin, Code: TRL	LK003230 LK003235	1 vi 5 vi	
HBSS Solution				**HBSS**
Sterile calcium and magnesium free Hank's balanced salt solution (CMFHBSS), pH 7.4, as supplied in the NCIS kit; 1 x 500 ml. Store at 2-8°C.		LK003210	1 ea	
L-15 Media Powder				**L15NK**
Leibovitz L-15 media powder, a component of the NCIS kit. Reconstitute entire contents of pouch, QS to 1 liter with cell culture grade water, and 0.22 micron filter. Suitable for cell isolation and culture applications. Store at 2-8°C.		LK003250	1 ea	
Cell Strainers (Falcon)				**CELSTRNK**
Cell strainers (Falcon), components of the NCIS kit. Suitable for removal of tissue debris in cell isolation applications. Store at room temperature.		LK003265	5 ea	
Neutral Protease (Dispase®), Purified				**NPRO**
Chromatographically purified. A lyophilized powder. Store at 2-8°C.	≥4 units per mg dry weight	LS02100 LS02104 LS02108	10 mg 50 mg Bulk	
Neutral Protease, Partially Purified				**NPRO2**
Partially purified. A lyophilized powder. Store at 2-8°C.	≥0.1 units per mg dry weight	LS02109 LS02111 LS02112	1 gm 5 gm Bulk	

Cell Isolation Product List

Product		Catalog #	Size	Code
Ovalbumin				**OA**
Major protein of egg white, with a molecular weight of 43,000. A lyophilized powder. Store at 2-8°C.		LS003049 LS003048 LS003050	1 gm 5 gm Bulk	
Ovalbumin, Purified				**OAC**
Chromatographically purified. Major protein of egg white, with a molecular weight of 43,000. A dialyzed, lyophilized powder. Store at 2-8°C.		LS003056 LS003054 LS003052	100 mg 1 gm Bulk	
Papain, Suspension				**PAP**
Supplied as a 2X crystalline suspension in 50mM sodium acetate, pH 4.5. To insure full activity, the enzyme should be incubated in a solution containing 1.1mM EDTA, 0.067mM mercaptoethanol and 5.5mM cysteine-HCl for 30 minutes. It is recommended that the enzyme be 0.22 micron filtered after dissolution and prior to use. Store at 2-8°C. REQUIRES SPECIAL SHIPPING: ICE PACK	Activates to at least 20 units per mg protein	LS003124 LS003126 LS003127 LS003128	25 mg 100 mg 1 gm Bulk	
Papain, Lyophilized				**PAPL**
Supplied as a lyophilized powder prepared from a 2X crystalline suspension, Code: PAP. To insure full activity, the enzyme should be incubated in a solution containing 1.1mM EDTA, 0.067mM mercaptoethanol and 5.5mM cysteine-HCl for 30 minutes. It is recommended that the enzyme be 0.22 micron filtered after dissolution and prior to use. Store at 2-8°C.	Activates to at least 15 units per mg protein	LS003118 LS003119 LS003120 LS003122	25 mg 100 mg 1 gm Bulk	
PDS Kit, Papain Vial				**PAP2**
A component of the Papain Dissociation System, for use in the tissue dissociation method of Huettner, J.E., and Baughman, R.W.: *J. Neuroscience, 6,* 3044 (1986). Contains papain, L-cysteine, and EDTA. This material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 5 ml of EBSS or equivalent yields a solution at 20 units of papain per ml in 1mM L-cysteine with 0.5mM EDTA. Store at 2-8°C.	≥100 units per vial	LK003176 LK003178	1 vi 5 vi	
Papain Dissociation System				**PDS**
Set of five single use vials of papain and five single use vials of DNase, 100 ml of Earle's balanced salt solution (EBSS), and an inhibitor vial for use in the tissue dissociation method of Huettner, J.E., and Baughman, R.W.: *J. Neuroscience, 6,* 3044 (1986). Use tested by Worthington using new-born rat pup spinal cord. The package contains sufficient materials for dissociation of five separate tissue aliquots of up to 0.3-0.4 cm^3 each. Store at 2-8°C.		LK003150 LK003153	1 bx 3 bx	

Cell Isolation Product List

Product		Catalog #	Size	Code
Papain Dissociation System, Without EBSS				**PDS2**
Complete kit as described for product Code: PDS, but without the Earle's Balanced Salt Solution (EBSS). Store at 2-8°C.		LK003160 LK003163	1 bx 3 bx	
PDS Kit, DNase Vial				**D2**
A component of the Papain Dissociation System. This material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 0.5 ml of EBSS or equivalent yields a solution of 2000 units/ml of deoxyribonuclease (1 mg/ml). Store at 2-8°C.	≥1,000 units per vial	LK003170 LK003172	1 vi 5 vi	
PDS Kit, Inhibitor Vial				**OI-BSA**
Ovomucoid protease inhibitor and bovine serum albumin which is 0.22 micron filtered and lyophilized in autoclaved vials to contain 10 mg/ml each upon reconstitution with 32 ml of EBSS. Store at 2-8°C.	≥300 mg TRL inhibited per vial	LK003182	1 vi	
PDS Kit, EBSS Vial				**EBSS**
Earle's balanced salt solution (EBSS) as supplied in the Papain Dissociation System. Store at 2-8°C.		LK003188	1 vi	
Proteinase K				**PROK**
A lyophilized powder. Purified to remove DNase and RNase. Store at 2-8°C.	≥20 units per mg dry weight	LS004220 LS004222 LS004224 LS004226	25 mg 100 mg 1 gm Bulk	
Proteinase K, Solution, 20mg/ml				**PROKS**
A concentrated, ready to use liquid formulation. Proteinase K prepared at 20 mg/ml in 10 mM Tris-HCl, 1 mM calcium acetate, pH 7.5 containing 50% glycerol. DNase and RNase free. Store at -20°C	≥400 units per milliliter	LS004240 LS004242 LS004244	5 ml 25 ml Bulk	
Trypsin 2X Lyo				**TRL**
Supplied as a chromatographically purified, diafiltered and lyophilized powder. Store at 2-8°C. PROTECT FROM MOISTURE	≥180 units per mg protein	LS003702 LS003703 LS003704 LS003706	100 mg 1 gm Bulk Bulk	
Trypsin				**TRL3**
Supplied as a chromatographically purified, diafiltered and lyophilized powder. Store at 2-8°C. PROTECT FROM MOISTURE	≥180 units per mg protein	LS003708 LS003707 LS003709	100 mg 1 gm Bulk	

Cell Isolation Product List

Product		Catalog #	Size	Code
Trypsin, 0.22µ Filtered				**TRLS**
Trypsin chromatographically purified, diafiltered, (Code TRL3) filtered thorugh a 0.22 micron pore size membrane and lyophilized in sterile vials. This product is not tested for pyrogenicity. Store at 2-8°C.	≥180 units per mg protein (at least 10,350 BAEE/3,450 USP/NF u/mgP)	LS003736 LS003734 LS003738	50 mg 5x50 mg Bulk	
Trypsin, 2X, Sterile, Irradiated				**TRLVMF**
2X crystallized (Code: TRL), lyophilized, irradiated and tested for the absence of mycoplasma and extraneous virus according to 9 CFR113.53c. Each vial is filled to contain ≥100 mg. Store at 2-8°C.	≥180 units per mg protein (at least 10,350 BAEE/3450 USP/NF units per mg protein)	LS004454 LS004452 LS004458	100 mg 5x100 mg Bulk	
Trypsin, Purified, Sequencing Grade II				**TRSEQII**
Bovine trypsin that has been treated with L-(tosylamido-2-phenyl) ethyl chloromethyl ketone (TPCK) to inhibit contaminating chymotryptic activity and extensively purified to remove autolysis products. Supplied as a lyophilized powder. Store at -20°C. PROTECT FROM MOISTURE. REQUIRES SPECIAL SHIPPING: ICE PACK	≥150 units per mg protein (at least 8,625 BAEE/2875 USP/NF units per mg protein)	LS02115 LS02117 LS02118 LS02119	4x25 µg 4x100 µg Bulk 1 mg	
Trypsin, Modified, Sequencing Grade				**TRSEQZ**
Trypsin, treated with L-(tosylamido-2-phenyl) ethyl chloromethyl ketone to inhibit contaminating chymotryptic activity, chemically modified to promote stability and further purified to remove autolysis fragments, resulting in a highly stable trypsin product resistant to autolysis while retaining specificity. Store at -20°C PROTECT FROM MOISTURE. REQUIRES SPECIAL SHIPPING: ICE PACK.	≥150 units per mg protein (at least 8,625 BAEE/2875 USP/NF units per mg protein)	LS02120 LS02122 LS02124	4x25 µg 4x100 µg Bulk	
Trypsin, TPCK Treated				**TRTPCK**
A chromatographically purified, diafiltered, lyophilized powder that has been treated with L-(tosylamido-2-phenyl) ethyl chloromethyl ketone (TPCK) to inhibit contaminating chymotryptic activity [Kostka, V., and Carpenter, F.: *JBC, 239,* 1799 (1964)]. Store at 2-8°C. PROTECT FROM MOISTURE	≥180 units per mg protein (at least 10,350 BAEE/3,450 USP/NF u/mgP)	LS003740 LS003741 LS003744 LS003742	100 mg 500 mg 1 gm Bulk	

Cell Isolation Product List

Product		Catalog #	Size	Code
Trypsin, TPCK-Treated, Irradiated				**TRTVMF**
Chromatographically purified trypsin treated with L-(tosylamido-2-phenyl) ethyl chloromethyl ketone (TPCK) to inhibit contaminating chymotryptic activity according to Kostka, V., and Carpenter, F.H.: *JBC, 239,* 1799 (1964), Code: TRTPCK, lyophilized, irradiated and tested for the absence of mycoplasma and extraneous virus according to 9 CFR 113.53c. Each vial is filled to contain ≥100 mg. Store at 2-8°C.	≥180 units per mg protein	LS003750 LS003752	100 mg 5x100 mg	
Trypsin Inhibitor, Lima Bean				**LBI**
Fraction III of the preparation described by Fraenkel-Conrat, *et al., Arch. Biochem. Biophys., 37,* 393 (1952). Supplied as a dialyzed, lyophilized powder. Store at 2-8°C.	1mg inhibits at least 2.2 mg trypsin, Code TRL	LS002829 LS002830 LS002831	100 mg 1 gm Bulk	
Trypsin Inhbitor, Ovomucoid				**OI**
Mucoprotein and antitryptic factor of egg white described by Lineweaver and Murray, *J. Biol. Chem., 171,* 565 (1947). A dialyzed, dried powder. Store at 2-8°C.	1 mg inhibits at least 1.2 mg trypsin, Code: TRL	LS003085 LS003087 LS003086 LS003089	500 mg 1 gm 2 gm Bulk	
Trypsin Inhibitor, Soybean, Purified				**SI**
Chromatographically purified as described by Frattali, V., and Steiner, R.: *Biochem., 7,* 521 (1968). A dialyzed, lyophilized powder. Purity checked using SDS PAGE. Store at 2-8°C.	1 mg inhibits at least 1.2 mg trypsin, Code: TRL	LS003570 LS003571 LS003573	100 mg 1 gm Bulk	
Trypsin Inhibitor, Soybean				**SIC**
A partially purified acetone powder. Store at 2-8°C.	1 mg inhibits at least 0.75 mg trypsin, Code: TRL	LS003587 LS003590	1 gm Bulk	

Ordering Information

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Lakewood, New Jersey 08701

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