ExoLutE® PLASMA & SERUM
Exosome Isolation Kit

USER GUIDE

Catalog Number EX-03
Revision B

BEFORE FIRST USE

"PLEASE READ CAREFULLY AND FOLLOW ALL THE INSTRCUTION"

EVEN IF YOU FEEL YOU ARE FAMILIAR with the products.

Store at 4˚C upon arrival.

RESEARCH USE ONLY

Rosetta Exosome®

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TRADEMARKS

BEFORE FIRST USE

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I

Product information

- Background
- Reagents provided and storage condition
- Consumables and laboratory equipment to be supplied by user

Background

Extracellular vesicles, also known as exosomes and microvesicles, are nano-vesicles (30-1,000 nm in diameters) produced by most living cells on the earth and composed of a variety of cellular components including proteins, lipids, and nucleic acids that are originated from their parental cells. These are found abundantly in the body fluids such as the blood, urine, saliva, cerebrospinal fluid, and breast milk.

Currently, extracellular vesicles are recognized as important biological mediators for intercellular communication in a variety of physiological events, where extracellular vesicles shuttle their cargo between cells. Over the past decade, molecular composition in extracellular vesicles has been proved to be associated with certain diseases and treatment responses, indicating that extracellular vesicles hold a great promise for diagnostic and prognostic tools for various diseases, as well as for therapeutic targets. Despite their importance, extracellular vesicle isolation is still considered as a major challenge, since both conventional methods with one-dimensional separation and tools in the field have not been satisfactory for excluding contaminants in the final products.

The science team in Rosetta Exosome Inc. has developed a novel multi-dimensional extracellular vesicle isolation workflow. This workflow comprises unique extracellular vesicle isolation technologies combined with precisely tuned spin-based size-exclusion chromatography. Using the workflow, extracellular vesicles in various biological fluids can be rapidly and successfully purified in intact form with superior purity as compared with other methods. Moreover, each line of ExoLutE® Exosome Isolation Kit is specifically designed for corresponding biological fluids such as mammalian cell-conditioned media, the cerebrospinal fluids, urine, plasma, and serum. The ExoLutE® Exosome Isolation Kit is composed of all the necessary reagents and materials which permit a simple and reliable method to produce **HIGHLY PURE**, **CONCENTRATED**, and **INTACT extracellular vesicles** for basic researches, biomarker discoveries, and clinical applications.

IMPORTANT NOTE: All currently available 'ExoLutE® Exosome Isolation Kits' are designed for the purification of exosomes and microvesicles, collectively as extracellular vesicles!

Reagents provided and storage condition

Each unit in this kit (10 units/kit) is designed for purifying extracellular vesicles from 0.2 – 0.5 mL of the plasma or serum. All reagents included in ExoLutE® Exosome Isolation Kit are sterilized by 0.22 μm pore-sized filters (but without preservatives). Take care to keep the reagent sterile after use.

Table 2 ExoLutE® Plasma & Serum Exosome Isolation Kit: 10 reactions

Component [1,2]	Amount	Part number	Storage
Sol A	6 mL	EX01L01	4°C
Sol B	20 mL	EX01L02	4°C
Sol C	50 mL	EX01L03	4°C
Sol D	10 mL	EX03L02	4°C
Sol R	6 mL	EX01L04	4°C
Liporemoval column	10 EA	EX03C01	4°C
Spin-SEC column	10 EA	EX01C03	4°C
Column bottom cap	10 EA	EX01S03	RT[3]
Waste tube, 2 mL	10 EA	EX03S02	RT[3]

[1] Store the components at the designated condition for up to 12 months.
[2] The stability of the kit will not be affected at room temperature during shipping
[3] Room temperature.

Consumables and laboratory equipment to be supplied by the user

Unless otherwise, all materials are available through other major laboratory suppliers.

Consumables:

- 15 mL conical centrifuge tubes.
- 1.5 mL and 2.0 mL microcentrifuge tubes.
- 0.45 μm pore-sized filters (or 0.8 μm pore-sized filters).
- Common culture media such as DMEM and RPMI1640.

Equipment:

- Vortex mixer.
- Rocker or an equivalent instrument for 15 mL falcon tubes and microcentrifuge tubes.
- Bench-top microcentrifuges for 1.5 mL or 2.0 mL microcentrifuge tubes.
- Laboratory-scale centrifuge (**swinging-bucket rotor**, 3,000 *xg* or higher) and appropriate **adaptors** for 50 ml and 15 mL conical centrifuge tubes.
- Personal protection equipments (lab coat, gloves, and goggles).

II

(Optional) Lipoprotein depletion

- Procedure guides
- Lipoprotein depletion

Procedure guides

- To isolate extracellular vesicles from the plasma, it is generally **NOT** recommended that users isolate extracellular vesicles from heparin-treated plasma.
- Heparin-treated plasma has not been tested.
- Aliquots of fresh plasma or serum MUST be frozen rapidly in liquid nitrogen or on dry ice. Avoid repeated freezing-and-thawing.
- When user uses an aliquot of frozen plasma or serum, **rapidly** thaw the plasma or serum **at room temperature**.
- One set of this kit is designed for purifying extracellular vesicles from 0.2 - 0.5 mL of EDTA- or citrate-treated plasma or serum.
- For any larger volumes of the plasma or serum, use multiple units per sample.

Lipoprotein depletion (optional)

NOTE, Certain subtypes of EVs in plasma or serum could be depleted at this step. If presence of lipoproteins or other nanoparticles in the EV preparation is not critical, this step could be omitted.

1. Centrifuge 0.2 - 0.5 mL of plasma or serum at **2,000 *xg* for 10 minutes** and then transfer the supernatant to a new microcentrifuge tube (not provided).
2. Keep the plasma/serum at room temperature until necessary.
3. Invert **Liporemoval column** (Part number EX03C01) several times.
4. Snap off the break-away end of Liporemoval column and remove the top cap.
5. Place the column on a 2 mL waste tube (provided).
6. Centrifuge at **500 *xg* for 30 seconds**.
7. Decant the flow through in the waste tube, and then replace the column on the tube.
8. Apply **0.5 mL of distilled water** to the column.
9. Centrifuge at **500 *xg* for 30 seconds**.
10. Transfer the column on a new 2 mL microcentrifuge tube (not provided).
11. Apply 0.2 - 0.5 mL of plasma or serum to the column.
12. Stand for 1 minute and then centrifuge at **500 *xg* for 1 minute.**
13. Transfer the column in a new 2 mL microcentrifuge tube and re-apply the flow through to the column.
14. Repeat step 13 then transfer the flow through in a new 1.5 mL microcentrifuge tube.
15. Proceed to the next procedure.

III

ExoLutE® PLASMA & SERUM
Exosome Isolation Kit Protocol

- Pre-treatment of plasma or serum
- Extracellular vesicle enrichment
- Workflow

Pre-treatment of plasma or serum

1. Mix **0.8 mL of Sol D** and **7.2 mL RPMI1640** (or equivalent) medium in a clean container.
2. Place 7.5 - 7.8 mL of the mixture prepared above (for 0.5 - 0.2 mL of plasma/serum, respectively) to a new 15 mL conical tube.
3. Add 0.2 - 0.5 mL of plasma or serum (or lipoprotein depleted one) then mix well.
4. Incubate at room temperature for 1 hour with continuous shaking.
5. Proceed to the next procedure.

Extracellular vesicle enrichment

IMPORTANT! WARM UP ALL REAGENTS AND SAMPLES to room temperature before use.

1. Add 0.25 mL of Sol A then cap the tube tightly. Invert the tube for 10 times.
2. Add 1.0 mL of Sol B then cap the tube tightly. Invert the tube for 10 times.
3. Add 4.0 mL of Sol C then cap the tube tightly. Invert the tube for 10 times.
4. Place the tube on a rocker, and then shake the tube at RT for 15 minutes.
   NOTE. Cap the tube tightly to prevent leakage while shaking.
5. Loosen the tube cap, and then centrifuge at 3,000 *xg* for 20 minutes at room temperature.
   IMPORTANT! Use a swinging-bucket rotor.
6. **Start column preparation during the step 5.**
7. Decant supernatant and leave the tube upside down on a filter paper for 1 - 2 minutes to remove residual fluid.
8. Add **300 µL of Sol R** and **COMPLETELY resuspend** the pellet by pipetting.
9. Vortex the crude EV resuspension for 30 sec and leave the suspension at room temperature for 5 - 10 minutes.

Column preparation

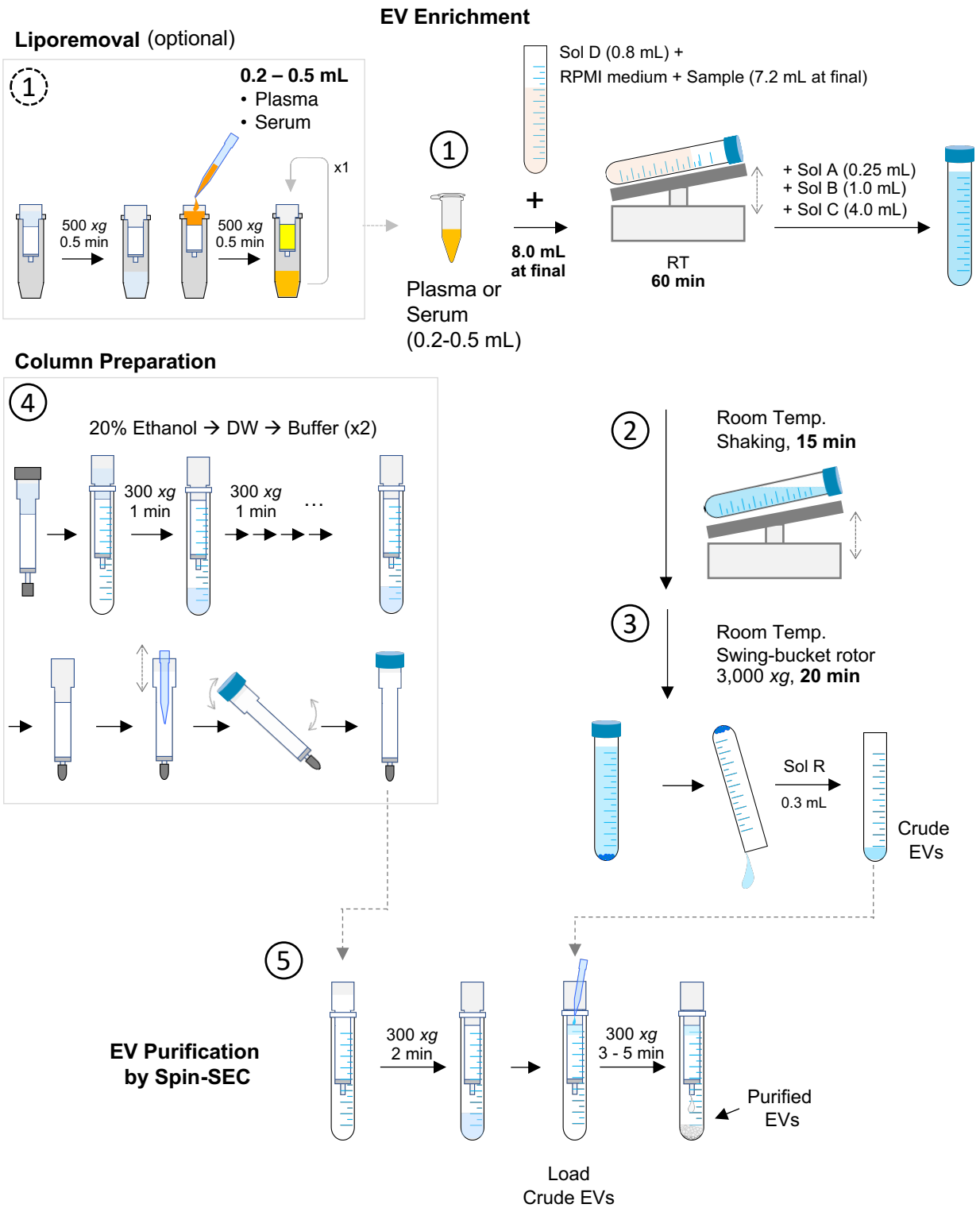
NOTE. It is **ESSENTIAL** to centrifuge the column in **a swinging-bucket** or horizontal rotor to make the sample to pass the column matrix evenly. **Set the instrument at maximum ACCELERATION and DECELERATION.**

1. Invert a Spin-SEC column several times to resuspend the white-colored matrix completely. Then stand it upright sitting in a 15 mL conical centrifuge tube (not provided).
2. Snap off the break-away end of the Spin-SEC column and remove the top cap. Place back the column in the 15 mL conical centrifuge tube.
3. Place the tube in a **swinging-bucket rotor** and centrifuge at **300 *xg* for 1 minute**.
4. Remove the flow through and replace the column in the 15 mL conical centrifuge tube.
5. Apply 2 mL of distilled water on the matrix in the column.
6. Centrifuge at **300 *xg* for 1 minute.**
7. Remove the flow through and replace the column in the 15 mL conical centrifuge tube.
8. Apply 2 mL of HEPES-buffered saline (HBS) or desired buffer on the matrix in the column.
9. Centrifuge at **300 *xg* for 1 minute.**
10. Repeat steps 6 - 8.
11. Remove the flow through and replace the column in the conical tube.
12. Place a cap tightly at the end of the column. Then apply 2 mL HBS (or desired buffer or fluid) on the matrix in the column.
13. Thoroughly mix the matrix by pipetting (~ 10 times).
14. Close the top of the column then invert the column to completely degas the matrix suspension.
15. Stand the column **UPRIGHT** sitting in the 15 mL conical tube at room temperature until use.

Purifying extracellular vesicles by spin-based size-exclusion column

1. Carefully remove caps on both ends of the column prepared above then place back in the 15 mL conical tube (for waste).
2. Place the tube in the **swinging-bucket rotor** and centrifuge at **300 *xg* for 2 minutes.**
3. Transfer the column in a **NEW** 15 mL conical centrifuge tube (not provided).
4. Slowly apply **300 µL of pellet suspension** prepared in the previous section to the **CENTER** of the matrix surface.
   IMPORTANT! AVOID OVERLOADING or UNDERLOADING.
5. Stand it upright for a minute.
6. Centrifuge at **300 *xg* for 3 - 5 minutes**.
7. Remove the column and transfer the collected highly purified extracellular vesicles to a NEW container.
8. (Optional) It is recommended to filter the purified EVs by **0.45 µm filter devices** (centrifugal filter device is better for recovery) for downstream analysis and application.
   NOTE. For long-term storage, flash freeze aliquots of the purified extracellular vesicles in **liquid nitrogen** then store at **-60˚C or below**.

Workflow


Liporemoval (optional)
1
0.2 – 0.5 mL
• Plasma
• Serum
x1
500 xg
0.5 min
500 xg
0.5 min
EV Enrichment
1
Plasma or
Serum
(0.2-0.5 mL)
Sol D (0.8 mL) +
RPMI medium + Sample (7.2 mL at final)
+
8.0 mL
at final
RT
60 min
+ Sol A (0.25 mL)
+ Sol B (1.0 mL)
+ Sol C (4.0 mL)
Column Preparation
4
20% Ethanol → DW → Buffer (x2)
300 xg
1 min
300 xg
1 min
2
Room Temp.
Shaking, 15 min
3
Room Temp.
Swing-bucket rotor
3,000 xg, 20 min
Sol R
0.3 mL
Crude
EVs
5
EV Purification
by Spin-SEC
300 xg
2 min
300 xg
3 - 5 min
Purified
EVs
Load
Crude EVs

Products currently available from Rosetta Exosome Inc.

Table 2 ExoLutE® Exosome Isolation Kits available from Rosetta Exosome Inc.

Recommended Kit	Catalog Number	Units/Kit
ExoLutE® Conditioned Medium	EX-01	10
ExoLutE® Urine	EX-02	10
ExoLutE® Plasma & Serum	EX-03	10

Table 3 Nanoparticle-free fetal bovine serum available from Rosetta Exosome Inc.

Recommended product	Catalog Number	Unit
Nanoparticle-free fetal bovine serum	ES-01	50 mL

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