FragIT™ kit

FOR RESEARCH
USE ONLY
www.genovis.com

STORE AT
+4-8°C

SmartEnzymes™

GENOVIS

INSTRUCTIONS FOR PRODUCTS

FragIT™kit Microspin 2 columns (A2-FR2-005)
Digestion and purification of up to 0.5 mg IgG

FragIT™kit Microspin 10 columns (A2-FR2-025)
Digestion and purification of up to 5 x 0.5 mg IgG

FragIT™kit Microspin 20 columns (A2-FR2-050)
Digestion and purification of up to 10 x 0.5 mg IgG

FragIT™kit Midispin (A2-FR2-100)
Digestion and purification of 1-10 mg IgG

FragIT™kit Maxispin (A2-FR2-1000)
Digestion and purification of 10-100 mg IgG

Quick Guide (only valid for FragIT kit Microspin)

- The Quick Guide (p. 3) is intended for experienced users. First time users are recommended to follow the detailed protocol (p. 8).
- Use lids and bottom caps during the incubation.
- Before centrifugation, remove the bottom cap and slightly open the lid.

Sample Preparation

- Prepare the antibody in 100-300 µl digestion buffer[1]. Max 0.5 mg IgG per column.

Last revised Dec 2019

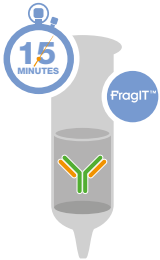
Digestion – FragIT™ Microspin

1 Equilibration

Equilibrate the column with 3 x 300 µl digestion buffer. Centrifuge at 200 x g for 1 min.

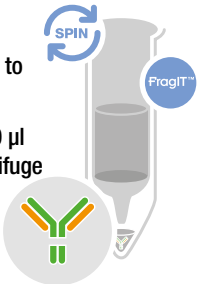
2 Digestion

- Add the antibody to the FragIT column.
- Cap the column and incubate at room temperature with end-over-end mixing for 15 min.

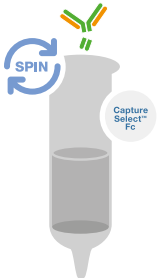


3 Collection

- Centrifuge at 1000 x g for 1 min to collect the antibody fragments.
- For maximum recovery, add 100 µl digestion buffer, invert and centrifuge at 1000 x g for 1 min.
- Repeat once.



Purification of F(ab')2 Fragments – CaptureSelect™ Fc Column

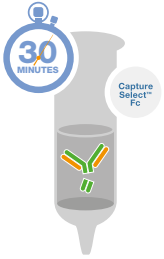


4 Equilibration

Equilibrate the CaptureSelect™ column with 3 x 300 µl binding buffer. Centrifuge at 200 x g for 1 min.

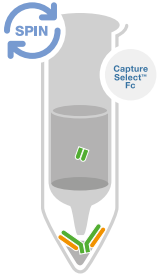
5 Binding of Fc

- Add the collected antibody fragments from step 3.
- Cap the column and incubate with end-over-end mixing for 30 min at room temperature.

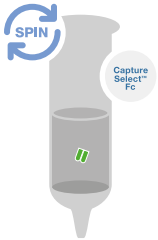


6 Collection of F(ab')2

- Centrifuge at 200 x g for 1 min to collect the F(ab')2 fragments.
- For maximum recovery, add 100 µl digestion buffer and centrifuge at 200 x g for 1 min.
- Repeat once and centrifuge at 1000 x g for 1 min in the final centrifugation step.



Elution of Fc Fragments – CaptureSelect™ Fc Column



7 Wash

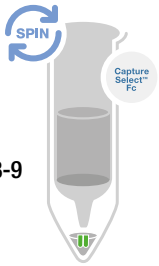
Wash the CaptureSelect™ Fc column with 3 x 300 µl binding buffer. Centrifuge at 200 x g for 1 min.

8 Elution of Fc

- Add 10 µl 1 M Tris, pH 8.0 to a collection tube.
- Add 100 µl 0.1 M Glycine, pH 3.0 to the CaptureSelect™ Fc column.
- Cap the column and invert manually a couple of times.

9 Collection of Fc

- Immediately, transfer the CaptureSelect™ Fc column to the collection tube and collect the Fc fragments by centrifugation at 200 x g for 1 min.
- For maximum recovery, repeat steps 8-9 and centrifuge at 1000 x g for 1 min.



PRODUCT DESCRIPTION

FragIT kit consists of an IgG digestion column, FragIT, and an affinity purification column, CaptureSelect™* Fc.

FragIT is a resin with FabRICATOR® enzyme covalently coupled to agarose beads for subunit generation of IgG. Pure F(ab')2 and Fc fragments are generated without the enzyme in the final preparation. The IgG is incubated with the FragIT resin and fragments are easily collected by a centrifugation step.

FragIT digests IgG at a specific site below the hinge region, and there is no risk of overdigestion if the incubation time is prolonged.
FragIT can be used with all commonly used buffers with pH ranging from 6.0 to 8.0. Optimization may be required.

FragIT digests all subclasses of human IgG as well as some classes of monkey, rat, rabbit and sheep IgG. It has limited activity on mouse IgG2a and IgG3 and for digestion and purification of fragments from mouse IgG2a and IgG3, FragIT™Z kit is recommended.

The CaptureSelect™* Fc column contains multi species Fc affinity matrix. A 13 kDa llama antibody fragment, recognizing Fc of multiple species with high affinity, is coupled to agarose beads.

The ligand is directed towards domains on the Fc part of IgG enabling binding and purification of IgG from a broad range of species, such as human, mouse, rat, rabbit, cow, horse, and sheep.

Content and Storage

FragIT kit contains two types of spin columns, one for IgG digestion and one for purification.

- The FragIT columns contain sufficient material to digest up to: 0.5 mg (Microspin), 10 mg (Midispin) or 100 mg (Maxispin) IgG. The resin is supplied in 20% EtOH with no preservatives added.
- CaptureSelect™* Fc column(s) – *Microspin:* One column contains sufficient material to purify up to 0.5 mg IgG. *Midispin:* One column contains sufficient material to purify up to 10 mg IgG. *Maxispin:* Two columns where each column includes sufficient material to purify up to 50 mg IgG. Supplied in 20% EtOH with no preservatives added.

FragIT kit is shipped cold and should be stored at +4-8°C upon arrival. **Do not freeze the product!**

FragIT kit is for R&D use only.

**Made with Thermo Scientific™ CaptureSelect™ resin from Thermo Fisher Scientific Inc. and its subsidiaries. Thermo Scientific and CaptureSelect are trademarks of Thermo Fisher Scientific Inc. and its subsidiaries.*

- Use lids and bottom caps during the incubation.
- Before centrifugation, remove the bottom cap and loosen the lid (do *not* remove the lid).
- Bottom caps for Midi- and Maxispin columns are included.
- Seal caps and lids of Midi- and Maxispin columns with parafilm during the incubation to prevent leakage.

Additional Materials Required

- Digestion buffer[1]: 10 mM sodium phosphate, 150 mM NaCl, pH 7.4.
- Binding buffer: 10 mM sodium phosphate, 150 mM NaCl, pH 7.4.
- Elution buffer: 0.1 M Glycine, pH 3.0.
- Neutralization buffer: 1 M Tris, pH 8.0.
- Collection tubes: 1.5-2 ml for Microspin, 15 ml for Midispin and 50 ml for Maxispin.

Sample Preparation

- Prepare the antibody in the digestion buffer[1] according to Table 1 below.

Table 1. *Preparation of antibodies*

Product Format	Microspin	Midispin	Maxispin
IgG in buffer	100-300 µl	0.5-2 ml	5-10 ml
Max amount IgG	0.5 mg	10 mg	100 mg

Digestion – FragIT™ Column

Protocol parameters for using the different product formats are given in Table 2.

1 Equilibration

- Break off the bottom cap of the FragIT column (save the cap for Microspin) and place the column in a collection tube. Loosen the lid.
- Centrifuge for 1 min to remove storage solution.
- Equilibrate the column by adding digestion buffer and centrifuge for 1 min.
- Repeat the equilibration step twice.
- Seal the spin column with the bottom cap.

2 Digestion

- Add the antibody to be digested in a volume of digestion buffer[1] according to Table 1.
- Seal the column with the top lid.
- Fully suspend the media, mix it by inversion and make sure there is a flow in the column.
- Incubate the column with end-over-end mixing at room temperature for for the time indicated in Table 2[2].

Table 2. *Protocol parameters for the different product formats*

Product Format	Microspin	Midispin	Maxispin
Storage solution removal			
Conical tubes	1.5-2 ml	15 ml	50 ml
Spin	200 x g	100 x g	100 x g
Equilibration			
Add buffer volume	300 µl (x3)	2.5 ml (x3)	10 ml (x3)
Spin	200 x g	100 x g	100 x g
Digestion			
Incubation time[2]	15 min	30 min	45 min
Collection of fragments			
Conical tubes	1.5-2 ml	15 ml	50 ml
Spin	1000 x g	100 x g	100 x g
Time	1 min	1 min	1 min
For max recovery			
Add buffer volume	100 µl (x2)	1 ml (x2)	5 ml (x2)
Spin	1000 x g	100 x g	100 x g

3 Collection of Fragments

- Remove the bottom cap and place the column in a collection tube. Loosen the top lid.
- Centrifuge the column for the time indicated in Table 2 to recover the fragments.

For Maximum Recovery of the Sample:

- Seal the column with the bottom cap.

- Add digestion buffer according to Table 2.
- Seal the column and invert it a couple of times.
- Remove the bottom cap and place the column in a collection tube. Loosen the top lid.
- Centrifuge the column for 1 min to elute the fragments.
- Repeat once.
- Pool the collected fractions.

Purification of F(ab')2 Fragments – CaptureSelect™ Fc Column

Protocol parameters for using the different product formats are given in Table 3.

4 Equilibration

- Break off the bottom seal of the CaptureSelect™ Fc column(s) (save the cap for Microspin) and place the column in a collection tube. Loosen the lid.
- Centrifuge for 1 min to remove storage solution.
- Equilibrate the column by adding binding buffer according to Table 3 and centrifuge for 1 min.
- Repeat the equilibration step twice.
- Seal the spin column with the bottom cap.

Table 3. *Protocol parameters for use of CaptureSelect™ Fc columns for the different product formats*

Product Format	Microspin	Midispin	Maxispin (per column)
Storage solution removal			
Conical tubes	1.5-2 ml	15 ml	50 ml
Spin	200 x g	200 x g	200 x g
Equilibration			
Add buffer volume	300 µl (x3)	3 ml (x3)	10 ml (x3)
Spin	200 x g	200 x g	200 x g
Binding of Fc			
Incubation time[2]	30 min	30 min	30 min
Collection of F(ab')2			
Conical tubes	1.5-2 ml	15 ml	50 ml
Spin	200 x g	200 x g	200 x g
For max recovery of F(ab')2			
Add buffer volume	100 µl (x2)	1 ml (x2)	2.5 ml (x2)
Spin 1	200 x g	200 x g	200 x g
Spin 2	1000 x g	200 x g	200 x g
Wash			
Add buffer volume	300 µl (x3)	3 ml (x3)	10 ml (x3)
Spin	200 x g	200 x g	200 x g
Elution of Fc			
Conical Tubes	1.5-2 ml	15 ml	50 ml
1M Tris in Conical Tubes	10 µl (x2)	100 µl (x2)	0.5 ml (x2)
0.1 M Glycine pH 3	100 µl (x2)	1 ml (x2)	5 ml (x2)
Spin 1	200 x g	200 x g	200 x g
Spin 2	1000 x g	200 x g	200 x g

5 Binding of Fc

- Immediately add the pooled collected fractions from step 3 to the CaptureSelect™ Fc column(s) and seal the column(s) with the top lid.
- Fully suspend the media, mix it by inversion and make sure there is a flow in the column.
- Incubate the column(s) with end-over-end mixing at room temperature for 30 min.

6 Collection of F(ab')2 Fragments

- Remove the bottom cap and place the column in a collection tube. Loosen the top lid.
- Centrifuge the column for 1 min to recover the F(ab')2 fragments.

For Maximum Recovery of F(ab')2 Fragments:

- Seal the spin column with the bottom cap.
- Add binding buffer to the column, seal the column and invert it a couple of times.
- Remove the bottom cap and place the column in a collection tube. Loosen the top lid.
- Centrifuge the column for 1 min according to Table 3 to collect the F(ab')2 fragments.
- Repeat addition of binding buffer once. Centrifuge for 1 min according to Table 3.
- Pool the collected F(ab')2 fragments.

Elution of Fc Fragments – CaptureSelect™ Fc Column

7 Wash

- Add binding buffer to the CaptureSelect™ Fc column according to Table 3.
- Centrifuge at 200 x g for 1 min.
- Repeat the wash twice.

8 Elution of Fc

- Prepare a collection tube with 1 M Tris, pH 8.0 of 0.1 x the elution volume according to Table 3.
- Seal the column with the bottom cap.
- Add 0.1 M Glycine, pH 3.0 to the CaptureSelect™ Fc column according to Table 3 and seal the column.
- Fully suspend the media by inverting the column a couple of times.
- Remove the bottom cap and place the column in a collection tube. Loosen the top lid.
- Centrifuge the column according to Table 3 for 1 min to elute the Fc fragments.

- Repeat elution (section 8) once. Centrifuge according to Table 3 for 1 min in the final centrifugation step.
- Pool the eluted Fc fractions.

Notes

1. *Other commonly used buffers at physiological pH and ionic strength can also be used.*
2. *The incubation time can be increased without overdigestion of the antibody.*

Quality Control

FragIT is tested to meet specification and to ensure lot-to-lot consistency.

FragIT is tested for absence of microbial contamination with blood agar plates, Sabouraud dextrose agar plates and fluid thioglycollate medium.

Related Products

FragIT™
Immobilized FabRICATOR®, digestion of IgG

FragIT™ Z
Immobilized FabRICATOR® Z, digestion of mouse IgG2a and IgG3

FragIT™ Z kit
Digestion of mouse IgG2a and IgG3, and purification of F(ab')2 and Fc fragments

GingisKHAN® Fab kit
Generation and purification of Fab fragments from hIgG1

FragIT™kit
Limited Use Label License: Research Use Only

The purchase of the ***IdeS*** enzyme from *Streptococcus pyogenes* (sold under the trade name FabRICATOR®) conveys to the purchaser the limited, non-transferable right to use the purchased amount of ***IdeS*** only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel.

Purchaser agrees to be bound by the following terms and restrictions:

1) A right is granted purchaser only for internal research purposes using ***IdeS*** for digesting an IgG and is not for use in commercial services of any kind, including, without limitation, reporting the result of purchaser's activities for a fee or other form of consideration.

2) ***IdeS*** will not be made available by purchaser to any third parties in any form, separately or in combination, for any monetary or other consideration or at no charge, except that ***IdeS*** may be made available to third parties who agree to be bound by all the terms and restrictions of this right for purposes of evaluation only.

3) ***IdeS*** and the digested IgG will not be used *in vivo* in humans.

4) Purchaser will not make commercial use of the ***IdeS*** unless it first secures a Sublicense Agreement from Genovis AB for such commercial use.

Genovis AB holds an exclusive world wide license to all patents derived from international publication WO03051914, including granted US Patent No US 7,666,582 regarding ***IdeS*** from *Streptococcus pyogenes* for biotechnical industrial applications which are neither therapeutic nor diagnostic, other than the following exception which is included within the license: digesting IgG in vitro in clinical samples for diagnostic purposes.

This license is defined in the intellectual property license agreement between Hansa Biopharma AB and Genovis AB.

The trademark FragIT™ is the property of Genovis AB.

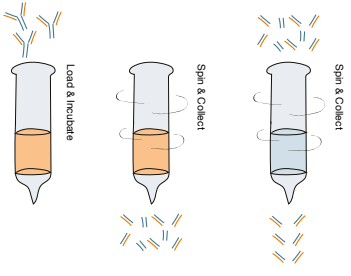
For research use only. Not intended for any animal or human therapeutic or diagnostic use.

All goods and services are sold subject to Genovis' General Terms and Conditions of Sale.

FabALACTICA® Fab kit

Easy Generation and Purification of Fab Fragments from Human IgG1

The FabALACTICA Fab kit consists of spin columns of Immobilized FabALACTICA for antibody digestion, and spin columns of CaptureSelect™* Fc resin for affinity binding of the Fc fragments. After digestion, the Fc fragments are captured in the affinity spin column and intact, pure Fab fragments are obtained in the flowthrough.



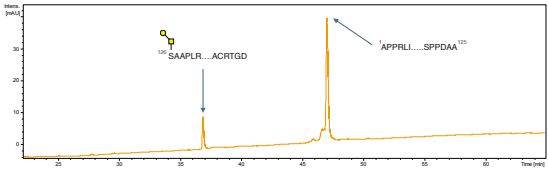
**Thermo Scientific™ CaptureSelect™ resin from Thermo Fisher Scientific Inc. and its subsidiaries. Thermo Scientific and CaptureSelect are trademarks of Thermo Scientific Inc. and its subsidiaries.*

OpeRATOR®

O-glycan-specific Endoprotease

OpeRATOR is a novel tool for analysis of mucin-type O-glycans on glycoproteins. The protein binds to O-glycans and digests the peptide backbone N-terminally of the S/T glycosylation sites.

- O-glycan-specific, mucin-type
- Requires O-glycans for activity
- Generates glycopeptides with O-glycans and allows for O-glycan profiling and site occupancy determination using mass spectrometry.



Erythropoietin (EPO) is a ~30 kDa glycoprotein with one core 1 O-glycan site. The protein was used here as a substrate to demonstrate the specific activity of the OpeRATOR protease. OpeRATOR hydrolyzed the protein N- terminally of the serine O-glycan site, and after reduction of disulfide bridges, the resulting two fragments were separated and intact mass was analyzed by Q-TOF MS using ESI.

USA & Canada

Genovis Inc.
245 First Street, Suite 1800
Cambridge, MA 02142
USA

Customer service: 1-617-444-8421
Order phone (toll free): 1-855-782-0084
Order fax: 1-858-524-3006
Email: orders.us@genovis.com

EMEA & Asia

Genovis AB
Box 790
SE-220 07 Lund
Sweden

Customer service: +46 46 10 12 30
Order phone: +46 46 10 12 30
Order fax: +46 46 12 80 20
Email: order@genovis.com