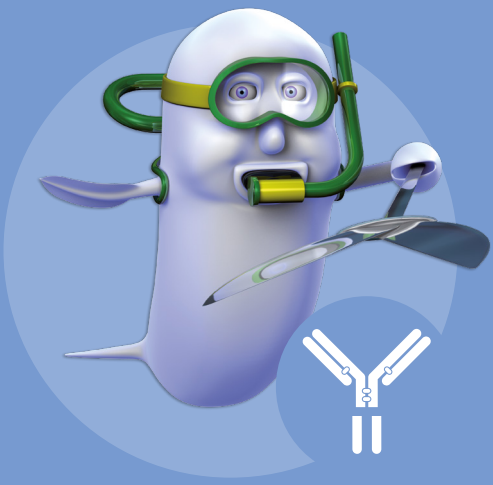
GENOVIS



FabRICATOR®

Fab2 Kit

STORE AT
+4-8°C

FOR RESEARCH USE ONLY

Instructions for Use

FabRICATOR® Fab2 Kit Microspin 0.5 mg (A2-FR2-005)
Process 0.5 mg IgG

FabRICATOR® Fab2 Kit Microspin 5 × 0.5 mg (A2-FR2-025)
Process 5 × 0.5 mg IgG

FabRICATOR® Fab2 Kit Microspin 10 × 0.5 mg (A2-FR2-050)
Process 10 × 0.5 mg IgG

FabRICATOR® Fab2 Kit Midispin 10 mg (A2-FR2-100)
Process 10 mg IgG

FabRICATOR® Fab2 Kit Maxispin 100 mg (A2-FR2-1000)
Process 100 mg IgG

DOWNLOAD INSTRUCTIONS FOR USE
www.genovis.com/ifu-A2-FR2

Immobilized Enzyme and Affinity Resin for Below Hinge Digestion of IgG and Purification of Fragments

FabRICATOR (IdeS) is an IgG-specific cysteine protease that digests antibodies at a single amino acid site below the hinge, generating homogenous F(ab')2 and Fc fragments. FabRICATOR Fab2 Kit consists of spin columns with FabRICATOR Immobilized resin for digestion of IgG, and spin columns with CaptureSelect™* Fc resin for affinity purification of F(ab')2 and Fc fragments. There is no risk of overdigestion if the incubation time is prolonged. Since FabRICATOR digests IgG under physiological reaction conditions, the immunoreactivity is preserved. FabRICATOR digests all subclasses of human, and some classes of monkey, rabbit, dog and sheep IgG. It has limited activity on mouse IgG2a and IgG3 – for digestion of these antibody species and purification of fragments, we recommend using FabRICATOR Z Fab2 Kit (A2-FZ2-005).

FabRICATOR is derived from *Streptococcus pyogenes* and expressed in *E. coli*. The enzyme contains a His-tag and has a molecular weight of 38 kDa.

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 of 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

FabRICATOR Fab2 Kit contains two components. The product box is shipped cold, and the two components should be stored at +4-8°C upon arrival. **Do not freeze the product!**

- **FabRICATOR Immobilized** is supplied in 20% ethanol with no preservatives added. One spin column contains sufficient material to digest 0.5 mg (Microspin), 10 mg (Midispin), or 100 mg (Maxispin) IgG.
- **CaptureSelect™ Fc column(s)** are supplied in 20% ethanol with no preservatives added. One column contains sufficient material to purify 0.5 mg (Microspin), 10 mg (Midispin), and 50 mg (Maxispin) IgG. Two columns are included in FabRICATOR Fab2 Kit Maxispin.

FabRICATOR Fab2 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.

QUALITY CONTROL

FabRICATOR Immobilized included in FabRICATOR Fab2 Kit is tested to meet the specifications and lot-to-lot consistency.

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

YOU MIGHT ALSO BE INTERESTED IN

FabRICATOR® Immobilized
Immobilized enzyme for below hinge digestion of IgG in spin columns

FabRICATOR® Z Immobilized
Immobilized enzyme for below hinge digestion of mouse IgG2a and IgG3 in spin columns

FabRICATOR® Z Fab2 Kit
Immobilized enzyme and affinity resin for below hinge digestion of mouse IgG2a and IgG3 and purification of fragments

FabALACTICA™ Fab Kit
Immobilized enzyme and affinity resin for above hinge digestion of human IgG1 and purification of fragments

Preparations

Important Information

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

Additional Materials Required

- Reaction buffer: 10 mM sodium phosphate, 150 mM NaCl, pH 7.4.[1]
- Binding buffer: 10 mM sodium phosphate, 150 mM NaCl, pH 7.4.
- Elution buffer: 100 mM glycine, pH 2.5.
- Neutralization buffer: 1 M Tris, pH 8.0.
- Centrifuge tubes: 1.5-2 ml for Microspin, 15 ml for Midispin and 50 ml for Maxispin.

Below Hinge Digestion of IgG

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

Sample Preparation
Prepare the antibody in the reaction buffer according to Table 1.

Table 1. Preparation of IgG

	Microspin	Midispin	Maxispin
IgG in buffer	100-300 µl	0.5-2 ml	5-10 ml
Amount IgG	0.5 mg	10 mg	100 mg

1. Equilibration

1.1 Break off the bottom cap of the FabRICATOR Immobilized column (save the cap for Microspin) and place the column in a centrifuge tube. Loosen the lid.

1.2 Centrifuge for 1 min to remove storage solution. Discard the flow-through.

1.3 Equilibrate the column by adding reaction buffer and centrifuge for 1 min. Discard the flow-through.

1.4 Perform step 1.3 two additional times.

1.5 Insert the bottom cap.

1. Other commonly used buffers at physiological pH and ionic strength can also be used.

2. Enzymatic Reaction

2.1 Add the antibody in a volume of reaction buffer according to Table 1.

2.2 Seal the column with the lid.

2.3 Fully suspend the media, mix by inversion and make sure there is a flow in the column.

2.4 Incubate the column with end-over-end mixing at room temperature for the time indicated in Table 2.[2]

Table 2. Protocol Parameters for the Different Product Formats

	Microspin	Midispin	Maxispin
Storage Solution Removal			
Centrifuge tubes	1.5-2 ml	15 ml	50 ml
Spin	200 × g	100 × g	100 × g
Time	1 min	1 min	1 min
Equilibration			
Add buffer volume	300 µl (×3)	2.5ml (×3)	10 ml (x3)
Spin	200 × g	100 × g	100 x g
Time	1 min	1 min	1 min
Enzymatic Reaction			
Incubation time[2]	15 min	30 min	45 min
Collection of Processed Material			
Centrifuge tubes	1.5-2 ml	15 ml	50 ml
Spin	1000 × g	100 × g	100 × g
Time	1 min	1 min	1 min
For Maximum Recovery			
Add buffer volume	100 µl (×2)	1 ml (×2)	5 ml (×2)
Spin	1000 × g	100 × g	100 × g
Time	1 min	1 min	1 min

3. Collection of Processed Material

3.1 Remove the bottom cap and place the column in a new centrifuge tube. Loosen the lid.

3.2 Centrifuge according to Table 2 to collect the processed material.

4. For Maximum Recovery of the Sample

4.1 Insert the bottom cap.

4.2 Add reaction buffer according to Table 2.

4.3 Seal the column with the lid and invert it a couple of times.

4.4 Remove the bottom cap and place the column in a new centrifuge tube. Loosen the lid.

4.5 Centrifuge according to Table 2 to collect the processed material.

4.6 Repeat steps 4.1-4.5.

4.7 Pool the collected fractions, including the sample from step 3.2.

2. The incubation time can be increased with no risk of overdigestion.

Purification of F(ab')2 Fragments

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

5. Equilibration

5.1 Break off the bottom seal of the CaptureSelect™ Fc column (save the cap for Microspin) and place the column in a centrifuge tube. Loosen the lid.

5.2 Centrifuge for 1 min to remove storage solution. Discard the flow-through.

5.3 Equilibrate the column by adding binding buffer according to Table 3 and centrifuge for 1 min. Discard the flow-through.

5.4 Perform step 5.3 two additional times.

5.5 Insert the bottom cap. Apply parafilm around the bottom cap to prevent leakage.

Table 3. Protocol Parameters for use of CaptureSelect™ Fc Columns for the Different Product Formats

	Microspin	Midispin	Maxispin (per column)
Storage Solution Removal			
Centrifuge tubes	1.5-2 ml	15 ml	50 ml
Spin	200 × g	200 × g	200 × g
Time	1 min	1 min	1 min
Equilibration			
Add binding buffer volume	300 µl (×3)	3 ml (×3)	10 ml (x3)
Spin	200 × g	200 × g	200 x g
Time	1 min	1 min	1 min
Binding of Fc			
Incubation time	30 min	30 min	30 min
Collection of F(ab')2			
Centrifuge tubes	1.5-2 ml	15 ml	50 ml
Spin	200 × g	200 × g	200 × g
Time	1 min	1 min	1 min
For Maximum Recovery of F(ab')2			
Add binding buffer volume	100 µl (×2)	1 ml (×2)	2.5 ml (×2)
Spin 1	200 × g	200 × g	200 × g
Spin 2	1000 × g	200 × g	200 × g
Time	1 min	1 min	1 min
Wash			
Add binding buffer volume	300 µl (×3)	3 ml (×3)	10 ml (×3)
Spin	200 × g	200 × g	200 × g
Time	1 min	1 min	1 min
Elution of Fc			
Centrifuge tubes	2 ml	15 ml	50 ml
1 M Tris in centrifuge tubes	20 µl (×3)	200 µl (×3)	1 ml (×3)
100 mM glycine pH 2.5	100 µl (×3)	1 ml (×3)	5 ml (×3)
Spin 1	200 × g	200 × g	200 × g
Spin 2 and 3	1000 × g	200 × g	200 × g
Time	1 min	1 min	1 min

6. Binding of Fc Fragments

6.1 Add the pooled collected fractions from step 4.7 to the CaptureSelect™ Fc column[3] and seal the column with the lid.

6.2 Fully suspend the media, mix by inversion and make sure there is a flow in the column.

6.3 Incubate the column with end-over-end mixing at room temperature for 30 min.

7. Collection of F(ab')2 Fragments

7.1 Remove the bottom cap and place the column in a new centrifuge tube. Loosen the lid.

7.2 Centrifuge according to Table 3 to collect the F(ab')2 fragments.

8. For Maximum Recovery of the Sample

8.1 Insert the bottom cap.

8.2 Add binding buffer according to Table 3.

8.3 Seal the column with the lid and invert it a couple of times.

8.4 Remove the bottom cap and place the column in a new centrifuge tube. Loosen the lid.

8.5 Centrifuge according to Table 3 to collect the F(ab')2 fragments.

8.6 Repeat steps 8.1-8.5.

8.7 Pool the collected F(ab')2 fragments, including the sample from step 7.2.

Elution of Fc Fragments

9. Wash

9.1 Add binding buffer to the CaptureSelect™ Fc column according to Table 3.

9.2 Centrifuge at 200 × g for 1 min.

9.3 Perform steps 9.1-9.2 two additional times

10. Elution of Fc Fragments

10.1 Prepare a new centrifuge tube with 1 M Tris, pH 8.0 according to Table 3.

10.2 Insert the bottom cap.

10.3 Add 100 mM glycine, pH 2.5 to the CaptureSelect™ Fc column according to Table 3 and seal the column with the lid.

10.4 Fully suspend the media by inverting the column a couple of times.

10.5 Remove the bottom cap and place the column in the centrifuge tube. Loosen the lid.

10.6 Centrifuge according to Table 3 to elute the Fc fragments.

10.7 Perform steps 10.1-10.6 two additional times.

10.8 Pool the eluted Fc fractions and make sure that the pH is neutralized.

3. Note: For FabRICATOR Fab2 Fab Kit Maxispin, two CaptureSelect™ columns are used in this section. Distribute the pooled collected fractions from step 4.7 evenly between the two columns.

USA & Canada
Genovis Inc.
10919 Technology Place Suite C, San Diego, CA 92127, USA
Phone: 1-855-782-0084 (toll free)
Fax: 1-858-524-3006

EMEA & Asia
Genovis AB
Box 4, SE-244 21 Kävlinge, Sweden
Phone: +46 46 10 12 30
Fax: +46 46 12 80 20

support@genovis.com
www.genovis.com

GENOVIS