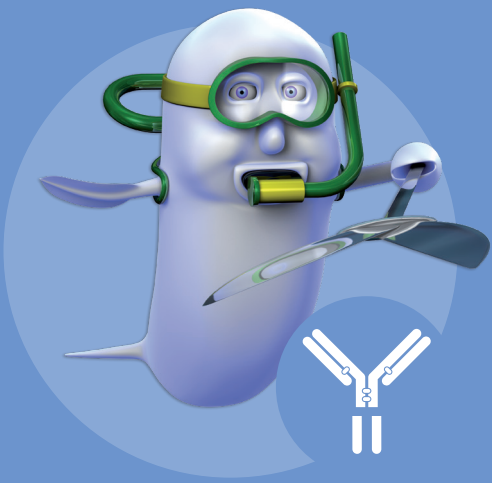
GENOVIS



FabRICATOR®

Immobilized

STORE AT
+4-8°C

FOR RESEARCH USE ONLY

Instructions for Use

FabRICATOR Immobilized
Microspin 2×0.5mg (A0-FR6-010)
Process 2×0.5mg IgG

FabRICATOR Immobilized
Microspin 5×0.5mg (A0-FR6-025)
Process 5×0.5mg IgG

FabRICATOR Immobilized
Microspin 10×0.5mg (A0-FR6-050)
Process 10 × 0.5 mg IgG

FabRICATOR Immobilized
Midispin 1-10mg (A0-FR6-100)
Process 1-10mg IgG

FabRICATOR Immobilized
Maxispin 10-100mg (A0-FR6-1000)
Process 10-100mg IgG

DOWNLOAD INSTRUCTIONS FOR USE
www.genovis.com/ifu-A0-FR6

Immobilized Enzyme for Below Hinge Digestion of IgG in Spin Columns

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. The FabRICATOR Immobilized spin columns contain FabRICATOR enzyme covalently coupled to agarose beads, for digestion of IgG without contaminating the final preparation with enzyme. 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, we recommend using FabRICATOR Z (A0-FRZ-020).

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

CONTENT AND STORAGE

The FabRICATOR Immobilized columns contain sufficient material to digest: 0.5 mg (Microspin), 10 mg (Midispin) or 100 mg (Maxispin) IgG per column. The resin is supplied in 20% EtOH with no preservatives added.

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

FabRICATOR Immobilized is for R&D use only.

QUALITY CONTROL

FabRICATOR Immobilized 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® Fab2 Kit
Immobilized enzyme and affinity resin for below hinge digestion of IgG and purification of fragments

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

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

Below Hinge Digestion of IgG

PREPARATIONS

Additional Materials Required

- Digestion buffer: 10 mM sodium phosphate, 150 mM NaCl, pH 7.4.[1]
- Collection tubes: 1.5-2 ml for Microspin, 15 ml for Midispin and 50 ml for Maxispin.

Sample Preparation

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

Table 1. Preparation of IgG

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

WORKFLOW

Important Information

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

1. Equilibration

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

1.2 Centrifuge for 1 min to remove storage solution.

1.3 Equilibrate the column by adding digestion buffer and centrifuge for 1 min.

1.4 Repeat the equilibration step two times.

1.5 Seal the spin column with the bottom cap.

1. Other commonly used buffers at physiological pH and ionic strength can also be used. Optimization may then be required.

2. Digestion

2.1 Add IgG in a volume of digestion buffer according to Table 1.

2.2 Seal the column with the top lid.

2.3 Fully suspend the media, mix it 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

Product Format	Microspin	Midispin	Maxispin
Storage Solution Removal			
Conical 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
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 × 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 Fragments

3.1 Remove the bottom cap and place the column in a collection tube. Loosen the top lid.

3.2 Centrifuge the column for 1 min to recover the fragments.

4. For Maximum Recovery of the Sample (Optional)

4.1 Seal the column with the bottom cap.

4.2 Add digestion buffer according to Table 2.

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

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

4.5 Centrifuge the column for 1 min to recover the fragments.

4.6 Repeat once.

4.7 Pool the collected fractions.

2. The incubation time can be increased without overdigestion of the antibody.

USA & Canada
Genovis Inc.
245 First Street, Suite 1800, Cambridge, MA 02142, USA
Phone: 1-855-782-0084 (toll free)
Fax: 1-858-524-3006

EMEA & Asia
Genovis AB
Box 790, SE-220 07 Lund, Sweden
Phone: +46 46 10 12 30
Fax: +46 46 12 80 20

support@genovis.com
www.genovis.com

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