



# FabRICATOR®

Immobilized

STORE AT

**+4-8°C**



FOR RESEARCH USE ONLY

## Instructions for Use

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

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

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

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

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



## 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')<sub>2</sub> 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 Immobilized (A0-FZ6-010).

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.

### 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

## 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.<sup>1</sup>
- 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.<sup>2</sup>

Table 2. Protocol Parameters for the Different Product Formats

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



**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-24421 Kävlinge, Sweden

Phone: +46 46 10 12 30

Fax: +46 46 12 80 20

[support@genovis.com](mailto:support@genovis.com)

[www.genovis.com](http://www.genovis.com)



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