SmartEnzymes™

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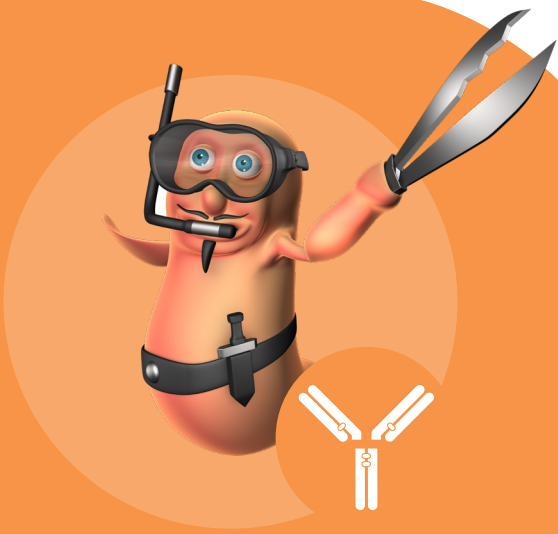

FabALACTICA®

Immobilized

STORE AT
+4-8°C

FOR RESEARCH USE ONLY

Instructions for Use

FabALACTICA® Immobilized
Microspin 2 × 0.5 mg (A0-AG6-010)
Process 2 × 0.5 mg hIgG1

FabALACTICA® Immobilized
Microspin 10 × 0.5 mg (A0-AG6-050)
Process 10 × 0.5 mg hIgG1

FabALACTICA® Immobilized
Midispin 5-10 mg (A0-AG6-100)
Process 10 mg hIgG1

FabALACTICA® Immobilized
Maxispin 50-100 mg (A0-AG6-1000)
Process 100 mg hIgG1

DOWNLOAD INSTRUCTIONS FOR USE
www.genovis.com/ifu-A0-AG6

www.genovis.com

Immobilized Enzyme for Above Hinge Digestion of Human IgG1 in Spin Columns

FabALACTICA (IgdE) is a cysteine protease that digests human IgG1 at a specific site above the hinge, generating intact and homogenous Fab and Fc fragments. The FabALACTICA Immobilized spin columns contain the FabALACTICA enzyme covalently coupled to agarose beads, for digestion of human IgG1 without contaminating the final preparation with enzyme. The digestion site on human IgG1 is …KSCDKT / HTCPPCP… There is no risk of overdigestion if the incubation time is prolonged. Since FabALACTICA digests human IgG1 under physiological reaction conditions, the immunoreactivity is preserved.

FabALACTICA is derived from *Streptococcus agalactiae* and expressed in *E. coli*. The enzyme contains a His-tag and has a molecular weight of 70 kDa.

CONTENT AND STORAGE

The FabALACTICA Immobilized columns contain sufficient material to digest: 0.5 mg (Microspin), 10 mg (Midispin) or 100 mg (Maxispin) human IgG1 per column. The resin is supplied in 20% ethanol with no preservatives added.

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

FabALACTICA Immobilized is for R&D use only.

QUALITY CONTROL

FabALACTICA Immobilized is tested to meet the specifications and lot-to-lot consistency.

FabALACTICA 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

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

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

FabDELLO™ Lyophilized
Lyophilized enzyme for above hinge digestion of human IgG1, including hinge-mutated IgG

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 caps and lids of Midi- and Maxispin columns with parafilm during the incubation to prevent leakage.

Additional Materials Required

- Reaction buffer: 150 mM sodium phosphate, pH 7.0.[1] To ensure efficient digestion, it is important to use the recommended reaction buffer.
- PBS: 10 mM sodium phosphate, 150 mM NaCl, pH 7.4.
- Centrifuge tubes: 1.5-2 ml for Microspin, 15 ml for Midispin and 50 ml for Maxispin.

Above Hinge Digestion of Human IgG1 in Spin Columns

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 hIgG1

	Microspin	Midispin	Maxispin
hIgG1 in buffer[1]	100 µl	1-2 ml[2]	5-10 ml[2]
Max amount hIgG1	0.5 mg	10 mg	100 mg

1. Equilibration

1.1 Break off the bottom cap of the FabALACTICA 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 the 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. Optimal activity is obtained in 100-150 mM sodium phosphate buffers at pH 6.5-7.5. Sodium chloride up to 150 mM can be added without affecting the enzyme activity.
2. The digestion efficiency is reduced if the antibody concentration is below 5 mg/ml.

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 overnight (16-18 h). A good mixing is important for optimal performance.

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)	2.5 ml (×3)	10 ml (×3)
Spin	200 × g	100 × g	100 × g
Time	1 min	1 min	1 min
Enzymatic Reaction			
Incubation time[3]	16-18 h	16-18 h	16-18 h
Collection of Processed Material			
Centrifuge tubes	1.5-2 ml	15 ml	50 ml
Spin	1000 × g	100 × g	100 × g
Time	1 min	2 min	2 min
For Maximum Recovery			
Add PBS 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 fragments.

4. For Maximum Recovery of the Sample

4.1 Insert the bottom cap.

4.2 Add PBS 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 fragments.

4.6 Repeat steps 4.1-4.5.

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

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

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