Immobilized

FabALACTICA®

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STORE AT
+4-8°C

SmartEnzymes™

GENOVIS

INSTRUCTIONS FOR PRODUCTS

Immobilized FabALACTICA® Microspin 2 columns
(A0-AG6-010) – Digestion of up to 2 × 0.5 mg IgG

Immobilized FabALACTICA® Microspin 10 columns
(A0-AG6-050) - Digestion of up to 10 × 0.5 mg IgG

Immobilized FabALACTICA® Midispin 1 column
(A0-AG6-100) - Digestion of 5-10 mg IgG

Immobilized FabALACTICA® Maxispin 1 column
(A0-AG6-1000) - Digestion of up to 100 mg IgG

Quick Guide (only valid for Microspin columns)

- The Quick Guide (p. 3) is intended for experienced users. First time users of all Immobilized FabALACTICA formats are recommended to follow the detailed protocol (p. 6).
- 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 µl digestion buffer at a maximum concentration of 5 mg/ml.

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Digestion
– Immobilized FabALACTICA® Microspin

1 Equilibration

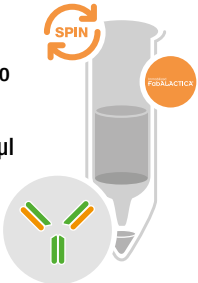
- Equilibrate the column with 3 x 300 µl digestion buffer. Centrifuge at 200 x g for 1 min.

2 Digestion

- Add the antibody to the column and cap the column.
- Incubate at room temperature with end-over-end mixing for 16-18 hours.

3 Collection

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



PRODUCT DESCRIPTION

Immobilized FabALACTICA is a resin with FabALACTICA (IgdE) enzyme covalently coupled to agarose beads for fragmentation of human IgG1 to generate Fab and Fc fragments without enzyme in the final preparation. The human IgG1 is incubated with the Immobilized FabALACTICA resin and the fragments are then easily collected by a centrifugation step.

Immobilized FabALACTICA digests human IgG1 under physiological reaction conditions thus preserving the immunoreactivity. The FabALACTICA enzyme digests human IgG1 above the hinge at ..KSCDKT /HTCPPCP.. The digestion is performed at room temperature overnight and there is no risk of overdigestion. Digestion can also be performed at 37 °C to decrease the incubation time, optimization is then required.

Content and Storage

The Immobilized FabALACTICA columns contain sufficient material to digest up to 0.5 mg (Microspin), 5-10 mg (Midispin) or 100 mg (Maxispin) hIgG1 per column. The resin is supplied in 20% EtOH with no preservatives added.

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

Immobilized FabALACTICA is for R&D use only.

- 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]: 150 mM sodium phosphate, pH 7.0.
- PBS buffer: 10 mM sodium phosphate, 150 mM NaCl, pH 7.4.
- 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[3]	100 µl[2,3]	1-2 ml	5-10 ml
Max amount IgG	0.5 mg	10 mg	100 mg

Digestion of hIgG1

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

1 Equilibration

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

2 Digestion

- Immediately add the hIgG1 in a volume reaction buffer according to Table 1.
- Seal the column with the top lid.
- Fully suspend the media, mix by inversion and **make sure there is a flow in the column.**

- Incubate the column by end-over-end mixing overnight (16-18 h) at room temperature. **A good mixing is important for optimal performance.**

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	16-18 h	16-18 h	16-18 h
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	2 min	2 min
For max recovery			
Add PBS 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 according to Table 2 to collect the fragments.

For Maximum Recovery of the Sample:

- Seal the spin column with the bottom cap.
- Add PBS buffer according to Table 2.
- Seal the column and invert 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 2 for 1 min to recover the fragments.
- Repeat the recovery steps once.
- Pool the collected fractions.

Notes

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 volume should be at least 100 µl / column, but can be increased up to 500 µl / column (Max 0.5 mg hIgG1).*
3. *The digestion efficiency is likely reduced if concentration is below 5 mg/ml.*

Quality Control

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

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

Related Products

FragIT™ kit
Generates and purifies F(ab')2 and Fc fragments from IgG

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

FabALACTICA® Fab kit
Generation and purification of intact Fab fragments from human IgG1

FabALACTICA®

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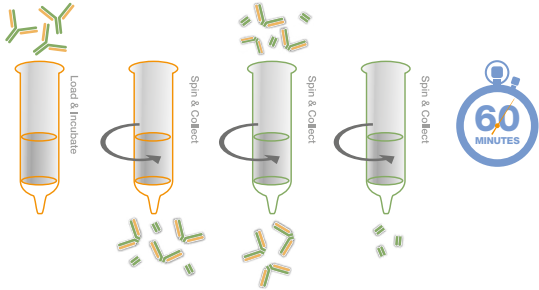
Genovis products are intended for research use only. They are not intended to be used for therapeutic or diagnostic purposes in humans or animals.

FragIT™ kit

Digestion of IgG and Purification of F(ab')2 and Fc Fragments

FragIT kit consists of an IgG digestion column, FragIT, and an affinity purification column, CaptureSelect™*. FragIT is a resin with FabRICATOR® enzyme covalently coupled to agarose beads for digestion of IgG to generate F(ab')2 and Fc fragments. After digestion, the fragments can easily be purified using the CaptureSelect™* column supplied in the kit.

- Digestion of IgG on a column.
- Purification of F(ab')2 and Fc fragments.



**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.*

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