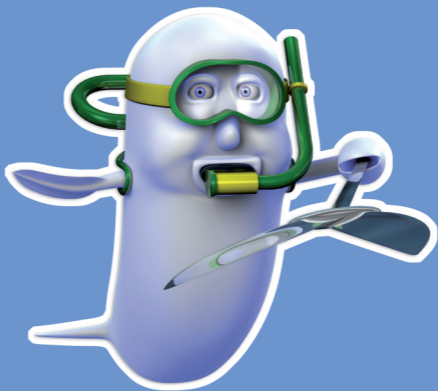


FragIT™

FOR RESEARCH
USE ONLY
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STORE AT
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SmartEnzymes™



GENOVIS

INSTRUCTIONS FOR PRODUCTS

FragIT™ Microspin 2 columns (A0-FR6-010)

Digestion of up to 2 × 0.5 mg IgG

FragIT™ Microspin 5 columns (A0-FR6-025)

Digestion of up to 5 × 0.5 mg IgG

FragIT™ Microspin 10 columns (A0-FR6-050)

Digestion of up to 10 × 0.5 mg IgG

FragIT™ Midispin 1 column (A0-FR6-100)

Digestion of up to 10 mg IgG

FragIT™ Maxispin 1 column (A0-FR6-1000)

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 formats of FragIT are recommended to follow the detailed protocol (p. 5).
- 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 100-300 µl digestion buffer. Max 0.5 mg IgG per column.

Digestion – FragIT™ Microspin

1 Equilibration

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



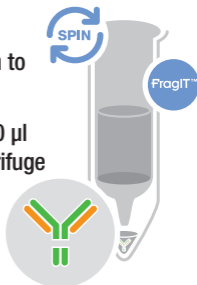
2 Digestion

- Add the antibody to the FragIT column and cap the column.
- Incubate at room temperature with end-over-end mixing for 15 min.



3 Collection

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



PRODUCT DESCRIPTION

FragIT is a resin with FabRICATOR® enzyme covalently coupled to agarose beads for subunit generation of IgG. Pure F(ab')₂ and Fc fragments are generated without the enzyme in the final preparation. The IgG is incubated with the FragIT resin and fragments are easily collected by a centrifugation step.

FragIT digests IgG at a specific site below the hinge region, and there is no risk of overdigestion if the incubation time is prolonged.

FragIT can be used with all commonly used buffers with a pH ranging from 6.0 to 8.0. Optimization may be required.

FragIT digests all subclasses of human IgG as well as some classes of monkey, rat, rabbit and sheep IgG. It has limited activity on mouse IgG2a and IgG3 and for digestion of these, FragIT™Z is recommended.

Content and Storage

The FragIT 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.

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

FragIT 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 buffer¹: 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 buffer¹ according to Table 1 below.

Table 1. Preparation of antibodies

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

Digestion of IgG on FragIT™ Column

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 for 1 min to remove storage solution.
- Equilibrate the column by adding digestion buffer and centrifuge for 1 min.
- Repeat the equilibration step two times.
- Seal the spin column with the bottom cap.

2 Digestion

- Add the antibody to be digested in a volume digestion buffer¹ according to Table 1.
- Seal the column with the top lid.
- Fully suspend the media, mix it by inversion and make sure there is a flow in the column.
- Incubate the column with end-over-end mixing at room temperature for the time indicated in Table 2².

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 ²	15 min	30 min	45 min
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	1 min	1 min
For max recovery			
Add 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 for the time indicated in Table 2 to recover the fragments.

For Maximum Recovery of the Sample:

- Seal the column with the bottom cap.
 - Add digestion buffer according to Table 2.
 - Seal the column and invert it a couple of times.
 - Remove the bottom cap and place the column in a collection tube. Loosen the top lid.
 - Centrifuge the column for 1 min to recover the fragments.
 - Repeat once.
 - Pool the collected fractions.
-

Notes

1. *Other commonly used buffers at physiological pH and ionic strength can also be used. Optimization may then be required.*
2. *The incubation time can be increased without overdigestion of the antibody.*

Quality Control

FragIT is tested to meet the specifications and lot-to-lot consistency.

FragIT 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

FragIT™

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Purchaser agrees to be bound by the following terms and restrictions:

1) A right is granted purchaser only for internal research purposes using **IdeS** for digesting an IgG and is not for use in commercial services of any kind, including, without limitation, reporting the result of purchaser's activities for a fee or other form of consideration.

2) **IdeS** will not be made available by purchaser to any third parties in any form, separately or in combination, for any monetary or other consideration or at no charge, except that **IdeS** may be made available to third parties who agree to be bound by all the terms and restrictions of this right for purposes of evaluation only.

3) **IdeS** and the digested IgG will not be used *in vivo* in humans.

4) Purchaser will not make commercial use of the **IdeS** unless it first secures a Sublicense Agreement from Genovis AB for such commercial use.

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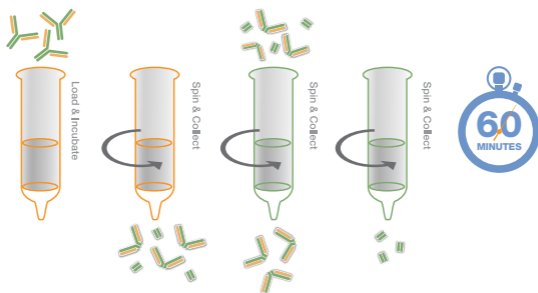
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FragIT™ kit

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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