

## INSTRUCTIONS

Version 15.1.2

Instructions for product no  
A2-FR2-100      2 columns      Cleavage and purification of up to 10 mg IgG

### Content and storage

FragIT<sup>™</sup> MidiKit contains 2 spin columns;

- One FragIT<sup>™</sup> MidiSpin column with sufficient material to digest 10 mg IgG. It is supplied in 20% EtOH with no preservatives added.
- One CaptureSelect<sup>®</sup> MidiSpin column, with sufficient material to purify up to 10 mg IgG. It is supplied in 20% EtOH with no preservatives added.

FragIT<sup>™</sup> MidiKit is shipped on ice and should be stored at +4-8°C upon arrival. Do not freeze the product!

### Product Description

FragIT<sup>™</sup> is a resin with FabRICATOR<sup>®</sup> enzyme covalently coupled to agarose beads for subunit fragmentation of IgG to generate pure F(ab')<sub>2</sub> and Fc fragments without contaminating the final preparation with enzyme. IgG is simply incubated with the FragIT<sup>™</sup> resin and fragments are then easily collected by a centrifugation step.

FragIT<sup>™</sup> cleaves IgG at one specific site below the hinge region and thereby eliminating the risk of over digestion if the incubation time is increased. FragIT<sup>™</sup> can be used with all commonly used buffers with pH ranging from 6.0 to 8.0. Optimization may be required.

FragIT<sup>™</sup> cleaves all subclasses of human, monkey, rabbit and sheep IgG but only subclass IgG2a and IgG3 of mouse IgG. Fragmentation of Mouse IgG2a with FragIT<sup>™</sup> requires significantly longer incubation time as compared to with human IgG.

The CaptureSelect<sup>®</sup> columns contains multi species Fc affinity matrix\* and is an agarose bead with a 13 kDa lama antibody fragment recognizing IgG of multiple species with high affinity. The used ligand is directed towards domains on the Fc part of IgG that enable purification of IgG of, amongst others, human, mouse, bovine, rabbit, rat, goat, horse, and sheep.

### Quality Control FragIT<sup>™</sup>

FragIT<sup>™</sup> is tested to ensure lot-to-lot consistency.

FragIT<sup>™</sup> is tested for absence of microbial contamination with blood agar plates, Sabouraud dextrose agar plates and fluid thioglycollate medium.

### Additional Materials Required

- Cleavage buffer: 10mM sodium phosphate, 150mM NaCl, pH 7.4 or similar with pH ranging from 6.0-8.0.
- Binding buffer: 10mM sodium phosphate, 150mM NaCl, pH 7.4.
- Collection tubes: 15 ml collection tubes.
- Parafilm
- Elution of Fc-Fragments: 0.1M Glycine pH 3.0 and 1M Tris pH 8.0.

### Method

- Lids and bottom caps are used during the incubation.
- Before centrifugation remove the bottom cap and slightly open the lid ~90° counter clockwise.

### Fragmentation

1. Make sure your antibody is in cleavage buffer (See Additional Material Required above).
2. Break off the bottom seal of the FragIT<sup>™</sup> column and slightly open the lid ~90° counter clockwise.
3. Place the column in a 15 ml collection tube.
4. Centrifuge the column at 100×g for 1min to remove storage solution.

*CaptureSelect<sup>®</sup> is a trademark of BAC BV Netherlands*

5. Equilibrate the column by adding 2.5 ml cleavage buffer.
6. Centrifuge the column at 100×g for 1 min.
7. Repeat steps 5 and 6 two times.
8. Put on the bottom cap on the column. Apply parafilm around the bottom cap to make sure there is no leakage.
9. Immediately add the IgG to be cleaved in a volume of 0.5-2.0 ml at a maximal concentration of 20 mg/ml in cleavage buffer.
10. Seal the column with the top lid. Apply parafilm around the top lid to make sure there is no leakage.
11. Take care to fully suspend the media manually and make sure it is flowing in the column.
12. Incubate the column by end-over-end mixing for 30 min in room temperature. The incubation time can be increased without over digestion of the IgG. \*For digestion of mouse IgG2a the incubation time needs to be significantly increased, see note below.
13. Remove the top lid and the bottom cap.
14. Place the column in a 15 ml collection tube.
15. Centrifuge the column at 100×g for 1 min to elute the sample.

For maximum recovery of your sample:

16. Place the column in a 15 ml collection tube.
17. Add 1.0 ml cleavage buffer.
18. Centrifuge the column at 100×g for 1 min to elute the sample.
19. Repeat steps 16-18 one more time.
20. Pool all the elution fractions.

**\*Digestion of Mouse IgG2a.**

For optimal fragmentation of mouse IgG2a the temperature can preferably be increased to 37°C and the incubation time needs to be optimized and can be increased to 6 – 48 hours.

**Purification**

1. Break off the bottom seal of the CaptureSelect® column and slightly open the lid ~90° counter clockwise.
2. Place the column in 15 ml collection tubes.
3. Centrifuge the column at 200×g for 1 min to remove storage solution.
4. Equilibrate the column with 3 ml binding buffer.
5. Centrifuge the column at 200×g for 1 min.
6. Repeat step 4 and 5 two times.
7. Seal the spin column with the bottom cap. Apply parafilm around the bottom cap to make sure there is no leakage.
8. Immediately add the pooled elution fractions on the CaptureSelect® column.
9. Re-seal the column with the top lid. Apply parafilm around the top lid to make sure there is no leakage.
10. Take care to fully suspend the media manually and make sure it is flowing in the columns.
11. Incubate the columns by end-over-end mixing at room temperature for 30min.
12. Remove the lid and the bottom cap.
13. Place the columns in new 15 ml collection tubes.
14. Centrifuge the columns at 200×g for 1 min to elute the sample.

*CaptureSelect® is a trademark of BAC BV Netherlands*

---

**Customer Service USA** Phone: 617-444-8421 | info@genovis.com | Genovis AB, 245 First Street, Cambridge, MA 02142, USA  
**Customer Service Europe** Phone: 0046 (0)46 10 12 30 | info@genovis.com | Genovis AB, Box 790, SE-220 07 Lund, Sweden  
**Support** support@genovis.com **Order** order@genovis.com | Fax: 0046 (0)46 12 80 20

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

For maximum recovery of your sample:

15. Add 1.0 ml binding buffer to each column.
16. Place the columns in new 15 ml collection tubes.
17. Centrifuge the columns at 200×g for 1min to elute sample.
18. Repeat step 15-17 one more time. At the final centrifugation centrifuge at 600×g for 1min.

### Elution of Fc fragments

To collect your Fc fragments, follow the below instructions.

1. Re-insert the bottom cap of the CaptureSelect® spin columns.
2. Add 1.0 ml 0.1M Glycine pH 3.0.
3. Re-seal the CaptureSelect® spin column with the lid.
4. Take care to fully suspend the media and invert the CaptureSelect® spin column manually a couple of times.
5. Open the lid and remove the bottom cap.
6. Place the column in a 15ml collection tube.
7. Centrifuge the CaptureSelect® spin column at 200×g for 1min to elute the sample.
8. Elution fractions are neutralized immediately with 0.1 volume 1M Tris pH 8.0.
9. Repeat step 1-8 one more time for maximum recovery. At the final centrifugation centrifuge at 1000×g for 1min.

### Limited Use Label License: Research Use Only

The purchase of the **IdeS** enzyme from *Streptococcus pyogenes* (sold under the trade name FabRICATOR®) conveys to the purchaser the limited, non-transferable right to use the purchased amount of **IdeS** only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel.

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.

For information on obtaining additional rights, please contact: [licensing@genovis.com](mailto:licensing@genovis.com)

©2015 Genovis AB. All rights reserved. Genovis AB holds the exclusive world wide license to all patents derived from international publication WO03051914, including granted US Patent No US 7,666,582 regarding **IdeS** from *Streptococcus pyogenes* for non-therapeutic use.

This license is defined in the intellectual property license agreement between Hansa Medical AB and Genovis AB.

*CaptureSelect® is a trademark of BAC BV Netherlands*

**Customer Service USA** Phone: 617-444-8421 | [info@genovis.com](mailto:info@genovis.com) | Genovis AB, 245 First Street, Cambridge, MA 02142, USA  
**Customer Service Europe** Phone: 0046 (0)46 10 12 30 | [info@genovis.com](mailto:info@genovis.com) | Genovis AB, Box 790, SE-220 07 Lund, Sweden  
**Support** [support@genovis.com](mailto:support@genovis.com) **Order** [order@genovis.com](mailto:order@genovis.com) | Fax: 0046 (0)46 12 80 20

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

FabRICATOR® is a trademark of Genovis AB

All goods and services are sold subject to Genovis' General Terms and Conditions of Sale.

*CaptureSelect® is a trademark of BAC BV Netherlands*

---

**Customer Service USA** Phone: 617-444-8421 | [info@genovis.com](mailto:info@genovis.com) | Genovis AB, 245 First Street, Cambridge, MA 02142, USA  
**Customer Service Europe** Phone: 0046 (0)46 10 12 30 | [info@genovis.com](mailto:info@genovis.com) | Genovis AB, Box 790, SE-220 07 Lund, Sweden  
**Support** [support@genovis.com](mailto:support@genovis.com) **Order** [order@genovis.com](mailto:order@genovis.com) | Fax: 0046 (0)46 12 80 20

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