FragITkit™

GENOVIS

INSTRUCTIONS

Version 15.1.2

A2-FR2-1000 1+2 columns Cleavage and purification of up to 100 mg IgG

Content and storage

FragIT™ MaxiKit contains 3 spin columns;

- One FragIT™ MaxiSpin column with sufficient material to digest 100 mg IgG. It is supplied in 20% EtOH with no preservatives added.
- Two CaptureSelect® MaxiSpin columns, each column include sufficient material to purify up to 50 mg IgG. It is supplied in 20% EtOH with no preservatives added.

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

Product Description

FragIT™ is a resin with FabRICATOR® enzyme covalently coupled to agarose beads for subunit fragmentation of IgG to generate pure $F(ab')_2$ and Fc fragments without contaminating the final preparation with enzyme. IgG is simply incubated with the FragIT™ resin and fragments are then easily collected by a centrifugation step.

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

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

The CaptureSelect® 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™

FragIT™ is tested to ensure lot-to-lot consistency.

FragIT™ 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: 50 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™ column and slightly open the lid ~90° counter clockwise.
3. Place the column in a 50 ml collection tube.

CaptureSelect® is a trademark of BAC BV Netherlands

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

4. Centrifuge the column at 100×g for 1min to remove storage solution.
5. Equilibrate the column by adding 10 ml cleavage buffer.
6. Centrifuge the column at 100×g for 1min.
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 5.0-10.0 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 45min 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 50 ml collection tube.
15. Centrifuge the column at 100×g for 1min to elute the sample.

For maximum recovery of your sample:

16. Place the column in a 50 ml collection tube.
17. Add 5.0 ml cleavage buffer.
18. Centrifuge the column at 100×g for 1min 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® columns and slightly open the lids ~90° counter clockwise.
2. Place the columns in 50 ml collection tubes.
3. Centrifuge the columns at 200×g for 1min to remove storage solution.
4. Equilibrate the columns with 10 ml binding buffer.
5. Centrifuge the columns at 200×g for 1min.
6. Repeat step 4 and 5 two times.
7. Seal the spin columns with the bottom caps. Apply parafilm around the bottom cap to make sure there is no leakage.
8. Immediately divide the pooled elution fractions equally between the two CaptureSelect® columns.
9. Re-seal the columns with the top lids. 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 lids and the bottom caps.
13. Place the columns in new 50 ml collection tubes.
14. Centrifuge the columns at 200×g for 1min to elute the sample.

For maximum recovery of your sample:

15. Add 2.5 ml binding buffer to each column.
16. Place the columns in new 50 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.
19. Pool the elution fractions from the two CaptureSelect® columns.

Elution of Fc fragments

To collect your Fc fragments, follow the below instructions.

1. Re-insert the bottom caps of the CaptureSelect® spin columns.
2. Add 5-10ml 0.1M Glycine pH 3.0 to each column.
3. Re-seal the CaptureSelect® spin columns with the lids.
4. Take care to fully suspend the media and invert the CaptureSelect® spin columns manually a couple of times.
5. Open the lids and remove the bottom caps.
6. Place the columns in 50ml collection tubes.
7. Centrifuge the CaptureSelect® spin columns 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.

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

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