FragITkit™

GENOVIS

INSTRUCTIONS

Version 15.1.2

Instructions for product no

A2-FR2-005	2 columns	Cleavage and purification of up to 0.5 mg IgG
A2-FR2-025	10 columns	Cleavage and purification of up to 5 x 0.5 mg IgG

Content and storage

FragIT™ MicroKit contains two different spin columns;

- FragIT™ MicroSpin column(s), each column include sufficient material to digest 0.5 mg IgG. It is supplied in 20% EtOH with no preservatives added.
- CaptureSelect® MicroSpin column(s), each column include sufficient material to purify up to 0.5 mg IgG. It is supplied in 20% EtOH with no preservatives added.

FragIT™ MicroKit 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: Micro centrifuge tubes (1.5-2 ml).
- 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 (save the cap) and slightly open the lid ~90° counter clockwise.

CaptureSelect® is a trademark of BAC BV Netherlands

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3. Place the column in a 1.5-2 ml collection tube.
4. Centrifuge the column at 200×g for 1min to remove storage solution.
5. Equilibrate the column by adding 300 µl cleavage buffer.
6. Centrifuge the column at 200×g for 1min.
7. Repeat steps 5 and 6 two times.
8. Put on the bottom cap on the column.
9. Immediately add the IgG to be cleaved in a volume of 100 µl at a maximal concentration of 5 mg/ml in cleavage buffer.
10. Seal the column with the top lid.
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 15min 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 1.5-2 ml collection tube.
15. Centrifuge the column at 1000×g for 1min to elute the sample.

For maximum recovery of your sample:

16. Place the column in a 1.5-2 ml collection tube.
17. Add 100 µl cleavage buffer.
18. Centrifuge the column at 1000×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 (save the cap) and slightly open the lids ~90° counter clockwise.
2. Place the column in 1.5-2 ml collection tubes.
3. Centrifuge the columns at 200×g for 1min to remove storage solution.
4. Equilibrate the columns with 300 µl binding buffer.
5. Centrifuge the columns at 200×g for 1min.
6. Repeat step 4 and 5 two times.
7. Seal the spin column with the bottom cap.
8. Immediately add the pooled elution fractions to the CaptureSelect® column.
9. Re-seal the columns with the top lids.
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 1.5-2 ml collection tubes.

14. Centrifuge the columns at 200×g for 1min to elute the sample.

For maximum recovery of your sample:

15. Add 100 µl binding buffer to the column.
16. Place the column in new 1.5-2 ml collection tube.
17. Centrifuge the column at 200×g for 1min to elute sample.
18. Repeat step 15-17 one more time. At the final centrifugation centrifuge at 1000×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 column.
2. Add 100 µl 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 1.5-2 ml 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.

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