



QA2-FR2-005 and QA2-FR2-025
FragIT™ Micro Kit Instructions for Use
Cleave and Purify up to 0.5mg IgG

Product Description

FragIT™ Micro Kit contains 2 spin columns – one for fragmentation of IgG and one for purification of F(ab')₂ fragments.

FragIT™ MicroSpin column contains FabRICATOR® enzyme covalently coupled to agarose beads for cleavage of IgG to pure F(ab')₂ and Fc fragments. IgG is first incubated with the FabRICATOR® agarose beads. F(ab')₂ and Fc fragments are then collected in a centrifugation step.

FabRICATOR® is a digestive enzyme that cleaves IgG at a specific site below the hinge region yielding pure F(ab')₂ and Fc fragments. Since FabRICATOR® cleaves only at one specific site, there is no risk of getting fragments other than F(ab')₂ and Fc even if the incubation time is increased.

FabRICATOR® cleaves all subclasses of human, monkey, rabbit, and sheep IgG but only mouse IgG2a and IgG3 subclasses. Cleavage of mouse IgG2a requires significantly longer incubation time as compared to cleavage of human IgG and may need to be optimized.

Optimal activity of FragIT™ is obtained at pH 6.6. It is possible to use a higher pH buffer and to increase the reaction time, but optimization is required.

The *CaptureSelect*® MicroSpin column contains a multi-species affinity matrix for Fc fragments comprised of agarose beads coupled to a 13kDa llama antibody fragment that recognizes Fc of multiple species with high affinity. Fc fragments can be eluted from this MicroSpin column using 200ul of a 0.1M glycine buffer, pH 3.0. Repeat the elution two additional times for maximum recovery.

Contents and Storage

FragIT™ MicroSpin column is supplied in 20% EtOH. No preservatives are added. One FragIT™ MicroSpin column is sufficient to cleave 0.5mg IgG.

FragIT™ MicroSpin is shipped on cold packs and should be stored at 4-8°C upon arrival.

CaptureSelect® MicroSpin column is supplied in 20% EtOH. No preservatives are added. One *CaptureSelect*® MicroSpin column is sufficient to purify up to 0.5mg IgG.

CaptureSelect® MicroSpin is shipped on cold packs and should be stored at 4-8°C upon arrival.

Quality Control

FabRICATOR® enzyme is tested to ensure lot-to-lot consistency.

FabRICATOR® enzyme is tested for sterility on blood agar plates, Sabaraud dextrose agar plates, and in thioglycolate broth.

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Additional Materials Required / Not Provided

- ✓ Cleavage buffer: 50mM sodium phosphate, 150mM NaCl, pH 6.6
- ✓ Binding buffer: 10mM sodium phosphate, 150mM NaCl, pH 7.4
- ✓ Collection tubes: Micro centrifuge tubes (1.5-2ml)

Procedure – IgG Cleavage

★ **Make sure your antibody is in cleavage buffer.**

1. Break off the bottom plastic cap of the FabRICATOR® spin column and slightly open the screw cap lid 90° counter-clockwise. **SAVE THE BOTTOM CAP!** Lids and bottom caps are used during the incubation step.
2. Place the spin column in a 1.5-2ml micro centrifuge tube.
3. Centrifuge the spin column at 200 x g for one (1) minute to remove storage solution.
4. Equilibrate the spin column with 300ul of cleavage buffer.
5. Centrifuge the spin column at 200 x g for one (1) minute.
6. Repeat steps 4 and 5 two more times.
7. Re-insert the bottom cap of the spin column.
8. Immediately add 100ul of IgG at a maximal concentration of 5mg/ml in cleavage buffer.
9. Re-seal the spin column with the lid.
10. Take care to stir and suspend the agarose beads manually and make sure the spin column will flow.
11. Incubate the spin column by end-over-end mixing at room temperature for 15 minutes. The incubation time can be increased without over digestion of the IgG. For cleavage of mouse IgG2a, the incubation time may need to be increased to 6 hours.
12. Open the spin column lid and remove the bottom cap.
13. Place the spin column in a 1.5-2ml micro centrifuge tube.
14. Centrifuge the spin column at 1,000 x g for one (1) minute to elute the sample.

For maximum recovery of sample:

15. Add an additional 100ul of cleavage buffer to the spin column.
16. Place the spin column in a 1.5-2ml micro centrifuge tube.
17. Centrifuge the spin column at 1,000 x g for one (1) minute to elute the sample.
18. Repeat steps 15-17 one more time.

Procedure – F(ab')₂ Purification

1. Break off the bottom plastic cap of the CaptureSelect® spin column and slightly open the screw cap lid 90° counter-clockwise. **SAVE THE BOTTOM CAP!** Lids and bottom caps are used during the incubation step.
2. Place the spin column in a 1.5-2ml micro centrifuge tube.
3. Centrifuge the spin column at 200 x g for one (1) minute to remove storage solution.
4. Equilibrate the spin column with 300ul of binding buffer.
5. Centrifuge the spin column at 200 x g for one (1) minute.
6. Repeat steps 4 and 5 two more times.
7. Re-insert the bottom cap of the spin column.
8. Immediately add the pooled elution fractions collected from the FabRICATOR® spin column.
9. Re-seal the spin column with the lid.
10. Take care to stir and suspend the agarose beads manually and make sure the spin column will flow.
11. Incubate the spin column by end-over-end mixing at room temperature for 30 minutes.
12. Open the lid and remove the bottom cap.
13. Place the spin column in a 1.5-2ml micro centrifuge tube.
14. Centrifuge the spin column at 200 x g for one (1) minute to elute the sample.

For maximum recovery of sample:

15. Add an additional 100ul of binding buffer to the spin column.
16. Place the spin column in a 1.5-2ml micro centrifuge tube.
17. Centrifuge the spin column at 200 x g for one (1) minute to elute the sample.
18. Repeat steps 15 and 16 one more time. For the final centrifugation, centrifuge at 600 x g for one (1) minute.