

FragIT™ Midi Kit, Cleave & purify up to 10 mg IgG

Instructions for product no
A2-FR2-100

Product Description

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

FragIT™ MidiSpin contains Fabricator covalently coupled to agarose beads for cleavage of IgG to generate pure F(ab')₂ and Fc fragments. IgG is incubated with the Fabricator agarose beads. F(ab')₂ and Fc are then collected by a centrifugation step. Since Fabricator is immobilized on agarose beads there is no need for extensive purification to remove the Fabricator enzyme.

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

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

Best activity of FragIT™ is obtained at pH 6.6. It is possible to use a buffer with a higher pH and increasing the reaction time. Optimization is required.

The CaptureSelect® MidiSpin column contains CaptureSelect® multi species Fc affinity matrix*. CaptureSelect multi species Fc affinity matrix is agarose beads with a 13 kDa Llama 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.

Content and storage

- ✓ Spin column containing FabRICATOR® covalently coupled to agarose beads.
- ✓ Spin column containing CaptureSelect Fc-specific matrix (Trademark of BAC BV, Netherlands).*

FragIT™ MidiSpin is supplied in 20% EtOH and no preservatives are added.

FragIT™ MidiSpin column contain sufficient FabRICATOR® coupled agarose beads to cleave 10 mg IgG.

FragIT™ MidiSpin is shipped on ice. FragIT™ MidiSpin should be stored at +4-8 °C upon arrival.

FragIT™ MidiSpin is for R&D use only.

CaptureSelect MidiSpin is supplied in 20% EtOH and no preservatives are added.

CaptureSelect MidiSpin column contain sufficient matrix to purify up to 10 mg IgG.

CaptureSelect MidiSpin is shipped on ice. CaptureSelect MidiSpin should be stored at +4-8 °C upon arrival.

CaptureSelect MidiSpin is for R&D use only.

*For more information about CaptureSelect® matrix see the web page of BAC BV (<http://www.captureselect.com>)

Quality Control FragIT™

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

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

FabRICATOR® is tested for absence of microbial contamination with blood agar plates, Sabaraud dextrose agar plates and fluid thioglycolate medium.

Additional Materials Required

- ✓ Cleavage buffer: 50mM sodium phosphate, 150mM NaCl, pH 6.6.
- ✓ Binding buffer: 10mM sodium phosphate, 150mM NaCl, pH 7.4.
- ✓ Collection tubes: 15 ml conical centrifuge tubes.

Method

Cleavage

- ✓ Make sure your antibody is in cleavage buffer (See *Additional Material Required* above).
- ✓ Lids and bottom caps are used during the incubation.
- ✓ An adaptor for 15 ml collection tubes for centrifugation is supplied.
- ✓ Before centrifugation remove the top and bottom cap.
 1. Remove the top lid and the bottom cap of the column.
 2. Place the column in a 15 ml collection tube by using the adaptor.
 3. Centrifuge the column at 100×g for 1min to remove storage solution.
 4. Equilibrate the column by adding 2.5 ml cleavage buffer.
 5. Centrifuge the column at 100×g for 1min.
 6. Repeat steps 4 and 5 two times.
 7. Put on the bottom cap on the column. Take care to seal it tightly by apply parafilm around the bottom cap to make sure there is no leakage.
 8. 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. For cleavage of mouse IgG2a see note below.
 9. 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 column.
 11. Incubate the column by end-over-end mixing for 30 min. The incubation time can be increased without over digestion of the IgG. For cleavage of mouse IgG2a see note below.
 12. Remove the top lid and the bottom cap.
 13. Place the column in a 15 ml collection tube by using the adaptor.
 14. Centrifuge the column at 100×g for 1min to elute the sample.

For maximum recovery of your sample:

15. Place the column in a 15 ml collection tube by using the adaptor.
16. Add 1.0 ml cleavage buffer.
17. Centrifuge the column at 100×g for 1min to elute the sample.
18. Repeat steps 15-17 one more time.

Cleavage of Mouse IgG2a.

Cleavage of mouse IgG2a has only been tested on FragIT™ MicroSpin columns. The incubation time needed here was 6 hours, which is significantly increased as compared to cleavage of human IgG. For optimal fragmentation the incubation time may need to be optimized for individual antibodies. Increasing the cleavage time increases the cleavage efficiency but with prolonged cleavage time the IgG may also be further fragmented.

Purification

- ✓ Break off the bottom plastic cap of the spin column.
- ✓ Lids and bottom caps are used during the incubation.
- ✓ Before centrifugation remove the bottom cap and slightly open the lid -90° counter clockwise.
 1. Break off the bottom cap of the spin column and slightly open the screw cap lid -90° counter clockwise.
 2. Place the column in a 15 ml collection tube.
 3. Centrifuge the column at 200×g for 1min to remove storage solution.
 4. Equilibrate the column with 3 ml binding buffer.
 5. Centrifuge the column at 200×g for 1min.
 6. Repeat step 4 and 5 two times.
 7. Seal the spin column with the bottom cap. Apply parafilm around the bottom to make sure there is no leakage.

8. Immediately add the pooled elutions fractions from the cleavage process with FragIT™.
9. Re-seal the column with the 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 column.
11. Incubate the column by end-over-end mixing at room temperature for 30 min.
12. Open the lid and remove the bottom cap.
13. Place the column in a new 15 ml collection tube.
14. Centrifuge the column at 200×g for 1min to elute the sample.

For maximum recovery of your sample:

15. Add 1 ml binding buffer.
16. Place the column in a new 15 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 600×g for 1min.

Product References

Mary H. Ryana, Diane Petrone, Jennifer F. Nemetha, Evan Barnathan, Lars Björck, Robert E. Jordan: *Proteolysis of purified IgGs by human and bacterial enzymes in vitro and the detection of specific proteolytic fragments of endogenous IgG in rheumatoid synovial fluid*, Molecular Immunology, October 2007.

Application note

FabRICATOR - perfect F(ab')₂ fragments in minutes