

INSTRUCTIONS

Version 15.1.2

Instructions for product no
A0-FR6-100 1 column Cleavage of up to 10 mg IgG

Content and storage

FragIT™ Midi contains;

- One FragIT™ MidiSpin column with sufficient material to digest 10 mg IgG. It is supplied in 20% EtOH with no preservatives added.

FragIT™ MidiSpin 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. Fragmentation of Mouse IgG2a with FragIT™ requires significantly longer incubation time as compared to with human IgG.

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.
- Collection tubes: 15 ml collection tubes.
- Parafilm

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 15 ml collection tube.
4. Centrifuge the column at 100×g for 1min to remove storage solution.
5. Equilibrate the column by adding 2.5 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 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.

Limited Use Label License: Research Use Only

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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.

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