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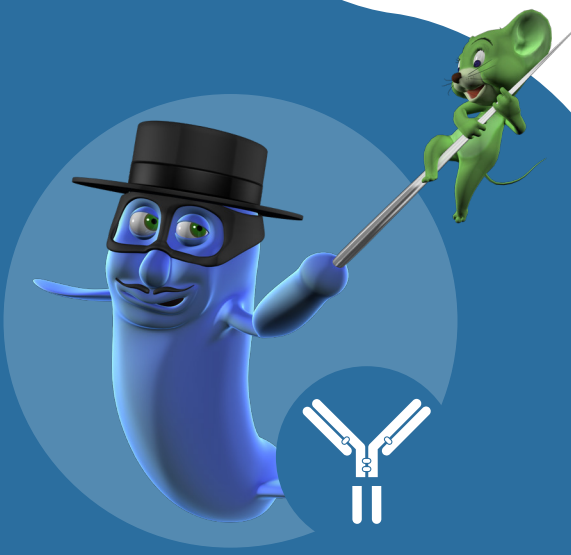

FabRICATOR®Z

Fab2 Kit

STORE AT
+4-8°C

FOR RESEARCH USE ONLY

Instructions for Use

FabRICATOR® Z Fab2 Kit
Microspin 0.5 mg (A2-FZ2-005)
Process 0.5 mg IgG

FabRICATOR® Z Fab2 Kit
Microspin 5 × 0.5 mg (A2-FZ2-025)
Process 5 × 0.5 mg IgG

DOWNLOAD INSTRUCTIONS FOR USE
www.genovis.com/ifu-A2-FZ2

Immobilized Enzyme and Affinity Resin for Below Hinge Digestion of Mouse IgG2a and IgG3 and Purification of Fragments

FabRICATOR Z (IdeZ) is a cysteine protease that digests mouse IgG2a and IgG3 at a single amino acid site below the hinge, generating homogenous F(ab')2 and Fc fragments. FabRICATOR Z Fab2 Kit consists of spin columns with FabRICATOR Z Immobilized for digestion of IgG, and spin columns with CaptureSelect™* Fc resin for affinity purification of F(ab')2 and Fc fragments. There is no risk of overdigestion if the incubation time is prolonged. Since FabRICATOR Z digests IgG under physiological reaction conditions, the immunoreactivity is preserved.

FabRICATOR Z is derived from *Streptococcus equi subsp. zooepidemicus* and expressed in *E. coli*. The enzyme contains a His-tag and the molecular weight is 36 kDa.

The CaptureSelect™ Fc column contains multi-species Fc affinity matrix. A 13 kDa llama antibody fragment, recognizing Fc of multiple species with high affinity, is coupled to agarose beads. The ligand is directed towards domains of the Fc part of IgG, enabling binding and purification of IgG from a broad range of species, such as human, mouse, rat, rabbit, cow, horse and sheep.

CONTENT AND STORAGE

FabRICATOR Z Fab2 Kit contains two components. The product box is shipped cold, and the two components should be stored at +4-8°C upon arrival. **Do not freeze the product!**

- **FabRICATOR Z Immobilized** is supplied in 20% ethanol with no preservatives added. One Microspin column contains sufficient material to digest 0.5 mg mouse IgG2a or IgG3.
- **CaptureSelect™ Fc Microspin column(s)** are supplied in 20% ethanol with no preservatives added. One column contains sufficient material to purify 0.5 mg mouse IgG2a or IgG3.

FabRICATOR Z Fab2 Kit is for R&D use only.

* Made with Thermo Scientific™ CaptureSelect™ resin from Thermo Fisher Scientific Inc. and its subsidiaries. Thermo Scientific and CaptureSelect are trademarks of Thermo Fisher Scientific Inc. and its subsidiaries.

QUALITY CONTROL

FabRICATOR Z Immobilized included in FabRICATOR Z Fab2 Kit is tested to ensure lot-to-lot consistency.

FabRICATOR Z Immobilized is tested for absence of microbial contamination with blood agar plates, Sabouraud dextrose agar plates and fluid thioglycolate medium.

YOU MIGHT ALSO BE INTERESTED IN

FabRICATOR® Immobilized
Immobilized enzyme for below hinge digestion of IgG in spin columns

FabRICATOR® Fab2 Kit
Immobilized enzyme and affinity resin for below hinge digestion of IgG and purification of fragments

FabRICATOR® Z Immobilized
Immobilized enzyme for below hinge digestion of mouse IgG2a and IgG3 in spin columns

GingisKHAN® Fab Kit
Lyophilized enzyme and affinity resin for above hinge digestion of human IgG1 and purification of Fab fragments

Preparations

Important Information

- Use lids and bottom caps during the incubation.
- Before centrifugation, remove the bottom cap and loosen the lid (do *not* remove the lid).

Additional Materials Required

- Digestion buffer: 10 mM sodium phosphate, 50 mM NaCl, pH 6.5.[1]
- Binding buffer: 10 mM sodium phosphate, 150 mM NaCl, pH 7.4.
- Elution buffer: 100 mM glycine, pH 2.5.
- Neutralization buffer: 1 M Tris, pH 8.0.
- Collection tubes: Microcentrifuge tubes (1.5-2 ml).

1. A digestion buffer with 50-150 mM NaCl, pH 6.5-7.5 can be used, but the digestion time needs to be increased with increasing NaCl concentrations and pH.

Below Hinge Digestion of IgG

Sample Preparation
Prepare the antibody to be digested in 100-300 µl digestion buffer per column, at a maximum concentration of 5 mg/ml.[1,2]

1. Equilibration

1.1 Break off the bottom cap of the FabRICATOR Z Immobilized column (save the cap) and place the column in a collection tube. Loosen the lid (do not remove the lid).

1.2 Centrifuge at 200 × g for 1 min to remove the storage solution.

1.3 Equilibrate the column by adding 300 µl digestion buffer and centrifuge at 200 × g for 1 min.

1.4 Repeat step 1.3 two times.

1.5 Seal the spin column with the bottom cap.

2. Digestion

2.1 Add the antibody to be digested in a volume of 100-300 µl digestion buffer.[2]

2.2 Seal the column with the top lid.

2.3 Fully suspend the media, mix it by inversion and make sure there is a flow in the column.

2.4 Incubate the column with end-over-end mixing at room temperature for 60 min.[3]

3. Collection of Fragments

3.1 Remove the bottom cap and place the column in a collection tube. Loosen the top lid.

3.2 Centrifuge the column at 1000 × g for 1 min to collect the fragments.

4. For Maximum Recovery of the Sample

4.1 Seal the column with the bottom cap.

4.2 Add 100 µl digestion buffer.

4.3 Seal the column and invert it a couple of times.

4.4 Remove the bottom cap and place the column in a collection tube. Loosen the top lid.

4.5 Centrifuge the column at 1000 × g for 1 min to collect the fragments.

4.6 Repeat steps 4.1-4.5 one time.

4.7 Pool the collected fractions.

2. The volume should be at least 100 µl/column, but it can be increased to up to 300 µl/column.
3. Increasing the temperature to up to 37°C will increase the digestion efficiency. The incubation time can be increased without overdigestion of the IgG.

Purification of F(ab')2 Fragments

5. Equilibration

5.1 Break off the bottom seal of the CaptureSelect™ Fc column (save the cap) and place the column in a collection tube. Loosen the lid.

5.2 Centrifuge at 200 × g for 1 min to remove the storage solution.

5.3 Equilibrate the column by adding 300 µl binding buffer and centrifuge at 200 × g for 1 min.

5.4 Repeat step 5.3 two times.

5.5 Seal the spin column with the bottom cap.

6. Binding of Fc Fragments

6.1 Add the pooled collected fractions from step 3 and 4 to the CaptureSelect™ Fc column and seal the columnwith the top lid.

6.2 Fully suspend the media, mix it by inversion and make sure there is a flow in the column.

6.3 Incubate the column with end-over-end mixing at room temperature for 30 min.

7. Collection of F(ab')2 Fragments

7.1 Remove the bottom cap and place the column in a collection tube. Loosen the top lid.

7.2 Centrifuge the column at 200 × g for 1 min to collect the F(ab')2 fragments.

8. For Maximum Recovery of the Sample

8.1 Seal the spin column with the bottom cap.

8.2 Add 100 µl binding buffer to the column.

8.3 Seal the column and invert it a couple of times.

8.4 Remove the bottom cap and place the column in a collection tube. Loosen the top lid.

8.5 Centrifuge the column for 1 min at 200 × g to collect the F(ab')2 fragments.

8.6 Repeat steps 8.1-8.5 one time. Centrifuge for 1 min at 1000 × g in the final centrifugation step.

8.7 Pool the collected fractions.

Elution of Fc Fragments

9. Wash

9.1 Add 300 µl binding buffer to the CaptureSelect™ Fc column.

9.2 Centrifuge at 200 × g for 1 min.

9.3 Repeat steps 9.1-9.2 two times.

10. Elution of Fc Fragments

10.1 Prepare a collection tube with 20 µl 1 M Tris, pH 8.0.

10.2 Seal the spin column with the bottom cap.

10.3 Add 100 µl 100 mM glycine, pH 2.5 to the CaptureSelect™ Fc column and seal the column.

10.4 Fully suspend the media by inverting the column a couple of times.

10.5 Remove the bottom cap and place the column in the collection tube. Loosen the top lid.

10.6 Centrifuge the column at 200 × g for 1 min to elute the Fc fragments.

10.7 Repeat steps 10.1-10.6 one time for maximum recovery. Centrifuge at 1000 × g for 1 min in the final centrifugation step.

10.8 Pool the eluted Fc fractions and make sure the pH is neutralized.

USA & Canada
Genovis Inc.
245 First Street, Suite 1800, Cambridge, MA 02142, USA
Phone: 1-855-782-0084 (toll free)
Fax: 1-858-524-3006

EMEA & Asia
Genovis AB
Box 790, SE-220 07 Lund, Sweden
Phone: +46 46 10 12 30
Fax: +46 46 12 80 20

support@genovis.com
www.genovis.com

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