SmartEnzymes™

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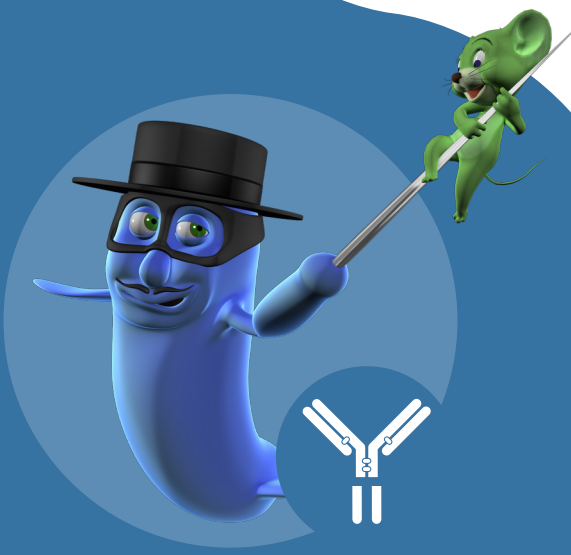

FabRICATOR®Z

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 resin 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 has a molecular weight of 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 Microspin** 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

- Reaction 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.
- Microcentrifuge tubes (1.5-2 ml).

1. A reaction buffer with 50-150 mM NaCl at pH 6.5-7.5 can be used, but the digestion time needs to be increased (2-24 h).

Below Hinge Digestion of Mouse IgG2a and IgG3 in Spin Columns

Sample Preparation
Prepare the antibody, 0.5 mg IgG, in 100-300 µl reaction buffer per column.

1. Equilibration

1.1 Break off the bottom cap of the FabRICATOR Z Immobilized column (save the cap) and place the column in a microcentrifuge tube. Loosen the lid.

1.2 Centrifuge at 200 × g for 1 min to remove the storage solution. Discard the flow-through.

1.3 Equilibrate the column by adding 300 µl reaction buffer and centrifuge at 200 × g for 1 min. Discard the flow-through.

1.4 Perform step 1.3 two additional times.

1.5 Insert the bottom cap.

2. Enzymatic Reaction

2.1 Add the prepared antibody solution to the spin column (0.5 mg IgG per column).

2.2 Seal the column with the lid.

2.3 Fully suspend the media, mix 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.[2]

3. Collection of Processed Material

3.1 Remove the bottom cap and place the column in a new microcentrifuge tube. Loosen the lid.

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

4. For Maximum Recovery of the Sample

4.1 Insert the bottom cap.

4.2 Add 100 µl reaction buffer.

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

4.4 Remove the bottom cap and place the column in a new microcentrifuge tube. Loosen the lid.

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

4.6 Repeat steps 4.1-4.5.

4.7 Pool the collected fractions, including the sample from step 3.2.

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 microcentrifuge tube. Loosen the lid.

5.2 Centrifuge at 200 × g for 1 min to remove the storage solution. Discard the flow-through.

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

5.4 Perform step 5.3 two additional times.

5.5 Insert the bottom cap.

6. Binding of Fc Fragments

6.1 Add the pooled collected fractions from step 4.7 (including the sample from step 3.2) to the CaptureSelect™ Fc column and seal the column with the lid.

6.2 Fully suspend the media, mix 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 new microcentrifuge tube. Loosen the lid.

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

8. For Maximum Recovery of the Sample

8.1 Insert the bottom cap.

8.2 Add 100 µl binding buffer to the column.

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

8.4 Remove the bottom cap and place the column in a new microcentrifuge tube. Loosen the lid.

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

8.6 Repeat steps 8.1-8.4, followed by centrifugation at 1000 × g for 1 min.

8.7 Pool the collected F(ab')2 fragments, including the sample from step 7.2.

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 Perform steps 9.1-9.2 two additional times.

10. Elution of Fc Fragments

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

10.2 Insert the bottom cap.

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

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 microcentrifuge tube. Loosen the lid.

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

10.7 Perform steps 10.1–10.6 two additional times. In step 10.6, centrifuge at 1000 × g for 1 min to elute the Fc fragments.

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

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