SmartEnzymes™

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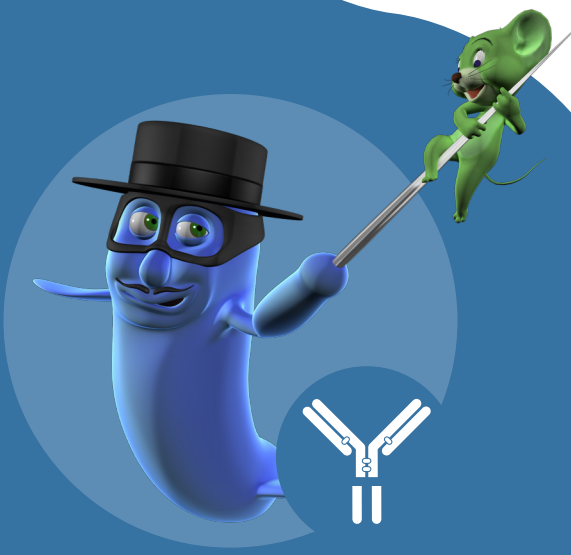

FabRICATOR®Z

Immobilized

STORE AT
+4-8°C

FOR RESEARCH USE ONLY

Instructions for Use

FabRICATOR® Z Immobilized
Microspin 2×0.5 mg (A0-FZ6-010)
Process 2×0.5 mg IgG

FabRICATOR® Z Immobilized
Microspin 5×0.5 mg (A0-FZ6-025)
Process 5×0.5 mg IgG

FabRICATOR® Z Immobilized
Microspin 10×0.5 mg (A0-FZ6-050)
Process 10×0.5 mg IgG

DOWNLOAD INSTRUCTIONS FOR USE
www.genovis.com/ifu-A0-FZ6

Immobilized Enzyme for Below Hinge Digestion of Mouse IgG2a and IgG3 in Spin Columns

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. The FabRICATOR Z Immobilized spin columns contain the FabRICATOR Z enzyme covalently coupled to agarose beads, for digestion of IgG without contaminating the final preparation with enzyme. 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. Most mouse IgG2a and IgG3 are digested by FabRICATOR Z within 2 hours, although the enzyme:antibody ratio and/or digestion time may need to be optimized for certain antibodies. FabRICATOR Z is also active on IgG from monkey, rabbit and sheep. For digestion of human IgG, we recommend using FabRICATOR Immobilized (A0-FR6-010).

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.

CONTENT AND STORAGE

The FabRICATOR Z Immobilized columns contain sufficient material to digest 0.5 mg mouse IgG2a or IgG3 per column. The resin is supplied in 20% ethanol with no preservatives added.

FabRICATOR Z Immobilized is shipped cold and should be stored at +4-8°C upon arrival. **Do not freeze the product!**

FabRICATOR Z Immobilized is for R&D use only.

QUALITY CONTROL

FabRICATOR Z Immobilized is tested to meet the specifications and lot-to-lot consistency.

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

YOU MIGHT ALSO BE INTERESTED IN

FabRICATOR® Z Fab2 Kit
Immobilized enzyme and affinity resin for below hinge digestion of mouse IgG2a and IgG3 and purification of fragments

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

FabALACTICA™ Immobilized
Immobilized enzyme for above hinge digestion of human IgG1 in spin columns

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]
- Microcentrifuge tubes (1.5-2 ml).

1. A digestion 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 in 100-300 µl reaction buffer per column. Max amount of IgG is 0.5 mg 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. Max 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. Increasing the temperature to 37°C will increase the digestion efficiency. The incubation time can be increased with no risk of overdigestion.

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