

# FabRICATOR<sup>®</sup>Z

Immobilized  
(Formerly FragIT Z)

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FOR RESEARCH  
USE ONLY  
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**SmartEnzymes**<sup>™</sup>

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## INSTRUCTIONS FOR PRODUCTS

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### **FabRICATOR Z Immobilized Microspin 2 × 0.5 mg**

Process 2 × 0.5 mg IgG (A0-FZ6-010)

### **FabRICATOR Z Immobilized Microspin 5 × 0.5 mg**

Process 5 × 0.5 mg IgG (A0-FZ6-025)

### **FabRICATOR Z Immobilized Microspin 10 × 0.5 mg**

Process 10 × 0.5 mg IgG (A0-FZ6-050)

## Quick Guide

- The Quick Guide (p. 3) is intended for experienced users. First time users are recommended to follow the detailed protocol (p. 5).
- Use lids and bottom caps during the incubation.
- Before centrifugation, remove the bottom cap and slightly open the lid.

## Sample Preparation

- Prepare the antibody in 100-300 µl digestion buffer. Max 0.5 mg IgG per column.

## Digestion – FabRICATOR Z Immobilized Microspin

### 1 Equilibration

- Equilibrate the column with  $3 \times 300 \mu\text{l}$  digestion buffer.  
Centrifuge at  $200 \times g$  for 1 min.



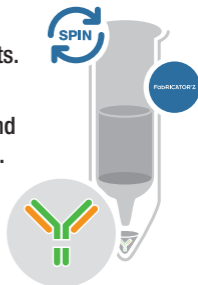
### 2 Digestion

- Add the antibody to the FabRICATOR Z Immobilized column.
- Cap the column and incubate at room temperature with end-over-end mixing for 60 min.



### 3 Collection

- Centrifuge at  $1000 \times g$  for 1 min to collect the antibody fragments.
- For maximum recovery, add  $100 \mu\text{l}$  digestion buffer, invert and centrifuge at  $1000 \times g$  for 1 min.
- Repeat once.



# PRODUCT DESCRIPTION

FabRICATOR Z Immobilized is a resin with FabRICATOR® Z enzyme covalently coupled to agarose beads for subunit generation of mouse IgG2a and IgG3. Pure F(ab')<sub>2</sub> and Fc fragments are generated without the enzyme in the final preparation. The IgG is incubated with the FabRICATOR Z Immobilized resin and fragments are then easily collected by a centrifugation step.

FabRICATOR Z Immobilized digests IgG at a specific site below the hinge region, and there is no risk of overdigestion if the incubation time is prolonged. The recommended buffer for FabRICATOR Z Immobilized is 10 mM sodium phosphate pH 6.5 with 50 mM NaCl<sup>1</sup>. The protocol may need optimization for individual antibodies.

## Content and Storage

Each FabRICATOR Z Immobilized Microspin column contains sufficient material to digest 0.5 mg mouse IgG2a or IgG3. The columns are supplied in 20% EtOH with no preservatives added.

FabRICATOR Z Immobilized Microspin is shipped cold and should be stored at +4-8°C upon arrival.

**Do not freeze the product!**

FabRICATOR Z Immobilized Microspin is for R&D use only.

- 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<sup>1</sup>: 10 mM sodium phosphate, 50 mM NaCl, pH 6.5.
- Collection tubes: Microcentrifuge tubes (1.5-2 ml).

## **Sample Preparation**

- Prepare the antibody to be digested in 100-300  $\mu$ l digestion buffer<sup>2</sup> per column. Max amount of IgG is 0.5 mg per column.

## Digestion - FabRICATOR Z Immobilized Column

### 1 Equilibration

- Break off the bottom cap (save the cap) of the FabRICATOR Z Immobilized column and place the column in a collection tube. Loosen the lid.
- Centrifuge at  $200 \times g$  for 1 min to remove the storage solution.
- Equilibrate the column by adding  $300 \mu\text{l}$  digestion buffer and centrifuge at  $200 \times g$  for 1 min.
- Repeat the equilibration step two times.
- Seal the spin column with the bottom cap.

### 2 Digestion

- Add the antibody to be digested in a volume of  $100\text{-}300 \mu\text{l}$  digestion buffer<sup>1,2</sup>. Max  $0.5 \text{ mg}$  IgG per column.
- Seal the column with the top lid.
- Fully suspend the media, mix it by inversion and make sure there is a flow in the column.
- Incubate the column with end-over-end mixing at room temperature for  $60 \text{ min}$ <sup>3</sup>.

### 3 Collection of Fragments

- Remove the bottom cap and place the column in a collection tube. Loosen the top lid.
- Centrifuge the column at 1000×g for 1 min to recover the fragments.

#### **For Maximum Recovery of the Sample:**

- Seal the spin column with the bottom cap.
- Add 100 µl digestion buffer.
- Seal the column and invert the column a couple of times.
- Remove the bottom cap and place the column in a collection tube. Loosen the top lid.
- Centrifuge the column at 1000×g for 1 min to recover the fragments.
- Repeat once.
- Pool the collected fractions.

#### **Notes**

1. A digestion buffer with 50-150mM NaCl at pH 6.5-7.5 can be used, but the digestion time needs to be increased (2-24 h) with increasing NaCl concentration and pH.
2. The volume should be at least 100 µl / column, but it can be increased to up to 300 µl / column. Max 0.5 mg IgG per 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.

## Quality Control

FabRICATOR Z Immobilized is tested to meet 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.

## Related Products

### **FabRICATOR Z Fab2 Kit**

Immobilized enzyme and affinity resin for below hinge digestion of mouse IgG2a and IgG3 and purification of fragments

### **FabRICATOR<sup>®</sup> Immobilized**

Immobilized enzyme for below hinge digestion of IgG in spin columns

### **FabRICATOR<sup>®</sup> Fab2 Kit**

Immobilized enzyme and affinity resin for below hinge digestion of IgG and purification of fragments

### **FabALACTICA<sup>®</sup> Immobilized**

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



**FabRICATOR® Z Immobilized**  
**Limited Use Label License: Research Use Only**

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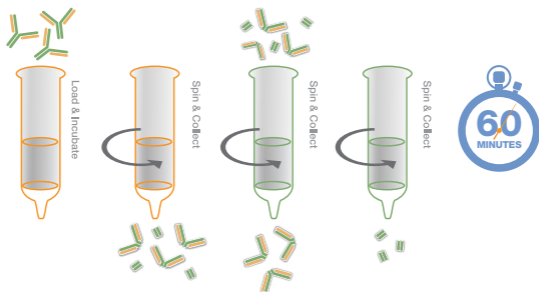
# OTHER PRODUCTS

## FabRICATOR® Fab2 Kit

Immobilized enzyme and CaptureSelect™ Fc for digestion and purification of IgG fragments

FabRICATOR Fab2 Kit consists of an IgG digestion column, FabRICATOR Immobilized, and an affinity purification column, CaptureSelect™\*. FabRICATOR Immobilized is a resin with FabRICATOR enzyme covalently coupled to agarose beads for digestion of IgG to generate F(ab')<sub>2</sub> and Fc fragments. After digestion, the fragments can easily be purified using the CaptureSelect™\* column supplied in the kit.

- Digestion of IgG on a column.
- Purification of F(ab')<sub>2</sub> and Fc fragments.



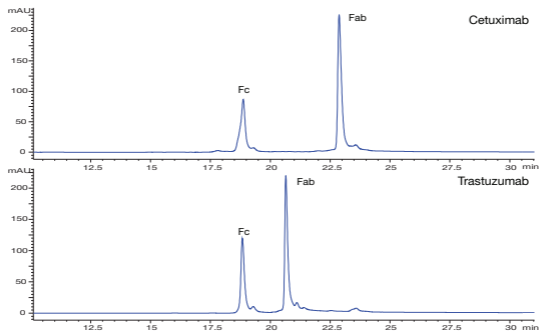
\* Made with Thermo Scientific™ CaptureSelect™ resin from Thermo Fisher Scientific Inc. and its subsidiaries. Thermo Scientific and CaptureSelect are trademarks of Thermo Fisher Scientific Inc. and its subsidiaries.

## FabALACTICA®

### Above hinge digestion of human IgG1

FabALACTICA is a cysteine protease that digests human IgG1 at a specific site in the upper hinge region, generating intact Fab and Fc fragments.

- Specific – one precise digestion site in the upper hinge of human IgG1.
- No need for reducing agents or co-factors.
- Easy to use, with no risk of overdigestion.





## **USA & Canada**

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