



Lyophilized



FOR RESEARCH USE ONLY

Instructions for Use

FabRICATOR® Lyophilized 2000 units (A0-FR1-020)Process 2 mg lgGFabRICATOR® Lyophilized 5000 units (A0-FR1-050)Process 5 mg lgGFabRICATOR® Lyophilized 5×5000 units (A0-FR1-250)Process 5×5 mg lgGFabRICATOR® Lyophilized 8×100 units (A0-FR1-008)Process 8×100 µg of lgGFabRICATOR® Lyophilized 96×100 units (A0-FR1-096)Process 96×100 µg of lgGFabRICATOR® Low Endotoxin 2000 units (A0-FR8-020)Process 2 mg lgGFabRICATOR® Low Endotoxin 5000 units (A0-FR8-050)Process 5 mg lgGFabRICATOR® Low Endotoxin 5000 units (A0-FR8-050)Process 5 mg lgGFabRICATOR® Validation Kit (A0-FR4-060)Process 3×2 mg lgG



Lyophilized Enzyme for Below Hinge Digestion of IgG

FabRICATOR (IdeS) is an IgG-specific cysteine protease that digests antibodies at a single amino acid site below the hinge, generating homogenous F(ab')2 and Fc fragments. The IgG is digested within 30 minutes, and there is no risk of overdigestion if the incubation time is prolonged. Since FabRICATOR digests IgG under physiological reaction conditions, the immunoreactivity is preserved. The best activity is obtained at 37°C, but digestion can also be performed at room temperature using a prolonged reaction time. FabRICATOR digests all subclasses of human, and some classes of monkey, rabbit, dog and sheep IgG. It has limited activity on mouse IgG2a and IgG3 – for digestion of these antibody species, we recommend using FabRICATOR Z (A0-FRZ-020).

FabRICATOR is cloned from *Streptococcus pyogenes* and expressed in *E. coli*. The enzyme contains a His-tag and has a molecular weight of 38 kDa.

UNIT DEFINITION

One unit FabRICATOR Lyophilized digests \ge 95% of 1 µg human IgG when incubated in PBS (10 mM sodium phosphate, 140 mM NaCl, 2.7 mM KCl), pH 7.4 at 37°C for 30 minutes.

CONTENT AND STORAGE

FabRICATOR Lyophilized is supplied lyophilized in PBS (10 mM sodium phosphate, 140 mM NaCl, 2.7 mM KCl), pH 7.4, with no preservatives added. FabRICATOR Lyophilized is shipped at ambient temperature and should be stored at -20°C upon arrival. After reconstitution, FabRICATOR Lyophilized is stable for at least 1 month at +4-8°C.

FabRICATOR is for R&D use only.

QUALITY CONTROL

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

FabRICATOR Lyophilized 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

Below hinge digestion of mouse IgG2a and IgG3

FabRICATOR[®] Immobilized

diaestion of IaG in spin columns

FabRICATOR® Fab2 Kit

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

Below Hinge Digestion of IgG in Vials

The workflow below is valid for the following FabRICATOR Lyophilized products:

- 2000 units (A0-FR1-020)
- 5000 units (A0-FR1-050)
- 5×5000 units (A0-FR1-250)
- Low Endotoxin 2000 units (A0-FR8-020)
- Low Endotoxin 5000 units (A0-FR8-050)
- Validation Kit (A0-FR4-060)

PREPARATIONS

Additional Materials Required

- Digestion buffer: see Table 1.
- For FabRICATOR Low Endotoxin, use endotoxin-free materials and solutions.

Sample Preparation

Prepare IgG in a compatible digestion buffer (Table 1). The final IgG concentration in the digestion reaction should be 0.5-10 mg/ml.

WORKFLOW

- 1. Prepare FabRICATOR
- 1.1 Reconstitute FabRICATOR according to Table 2.1

2. Add FabRICATOR

2.1 Add 1 unit FabRICATOR / 1 µg IgG. The final IgG concentration should be 0.5-10 mg/ml.

3. Digestion

3.1 Incubate for 30 min at 37°C.

 To prevent microbial contamination, sodium azide can be added to the solution to a final concentration of 0.02-0.05% (w/v).

Below Hinge Digestion of IgG in Plate Vials

The workflow below is valid for the following FabRICATOR Lyophilized products:

- 8 × 100 units (A0-FR1-008)
- 96×100 units (A0-FR1-096)

PREPARATIONS

Additional Materials Required

• Digestion buffer: see Table 1.

Sample Preparation

Prepare IgG in a compatible digestion buffer (Table 1). The final IgG concentration in the digestion reaction should be 1-2 mg/ml.

WORKFLOW

1. Add IgG to the FabRICATOR Plate Vial

1.1 Add the IgG solution directly to the lyophilized FabRICATOR plate vial (50-100 µl/vial). Each vial contains enough enzyme to digest 100 µg of antibody. Pierce the aluminum foil by using a standard pipette tip. Make sure to reconstitute all lyophilized material.

2. Digestion

2.1 Incubate for 30 min at 37°C.

Table 1. Buffers and pH² Compatible with FabRICATOR Digestion

Compatible Buffers	pН
Phosphate-buffered saline (PBS)	6.0-8.0
Tris buffer	7.0-8.0
MES buffer	5.5-6.5
HEPES buffer	7.0-8.0
Ammonium bicarbonate buffer	6.0-7.0
Sodium acetate buffer	6.0
Common formulation buffers ³	

Table 2. Recommended Volumes for Reconstitution of the FabRICATOR Enzyme

Product	Product Size	Reconstitution Volume
A0-FR1-020	2000 units	30µl ddH ₂ O
A0-FR1-050	5000 units	$75 \mu l ddH_2O$
A0-FR1-008	8×100 units	50-100 µl IgG/plate vial4
A0-FR1-096	96×100 units	50-100µl IgG/plate vial ^₄
A0-FR8-020	2000 units	30µl ddH ₂ O (LE)
A0-FR8-050	5000 units	75µl ddH ₂ O (LE)

- FabRICATOR enzyme is inactivated at pH < 5.0. Digestion at pH > 8.0 requires prolonged/optimized reaction conditions.
- FabRICATOR is compatible with several antibody formulation buffers.
- Reconstitution volume depends on IgG concentration. Each well contains enough enzyme to digest 100 µg of IgG.

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