

FabRICATOR®

FOR RESEARCH
USE ONLY
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STORE AT
-20°C



SmartEnzymes™



GENOVIS

INSTRUCTIONS FOR PRODUCTS

FabRICATOR® 2000 units (A0-FR1-020)

Digestion of up to 2.0 mg IgG

FabRICATOR® 5000 units (A0-FR1-050)

Digestion of up to 5.0 mg IgG

FabRICATOR® 5x5000 units (A0-FR1-250)

Digestion of up to 5 x 5.0 mg IgG

FabRICATOR® Validation kit (A0-FR4-060)

Validation kit, Digestion of up to 3 x 2.0 mg IgG

FabRICATOR® 8x100 units (A0-FR1-008)

Digestion of up to 8 x 100 µg of IgG (8 well strip)

FabRICATOR® 96x100 units (A0-FR1-096)

Digestion of up to 96 x 100 µg of IgG (96 well plate)

FabRICATOR® LE 2000 units (A0-FR8-020)

Low endotoxin (<0.04 EU/vial), Digestion of up to 2.0 mg IgG

FabRICATOR® LE 5000 units (A0-FR8-050)

Low endotoxin (<0.1 EU/vial), Digestion of up to 5.0 mg IgG

The quick guide is intended for FabRICATOR vials and experienced users. For FabRICATOR strips and plates, follow the protocol on page 8.

1 Prepare FabRICATOR®

Reconstitute FabRICATOR according to Table 3.



2 Add FabRICATOR®

Add 1 unit FabRICATOR / 1 μ g IgG.



3 Digestion

Incubate for 30 min at 37°C.



PRODUCT DESCRIPTION

FabRICATOR (IdeS) is an enzyme that digests IgG at one specific site just below the hinge region, resulting in F(ab')₂ and Fc fragments. Since FabRICATOR digests IgG at one specific site, there is no risk of overdigestion if the incubation time is prolonged.

FabRICATOR digests all subclasses of human IgG (Table 1) as well as some classes of monkey, rat, rabbit and sheep IgG. It has limited activity on mouse IgG2a and IgG3 and for digestion of these, FabRICATOR[®] Z (A0-FRZ-020) is recommended.

FabRICATOR digests IgG under physiological reaction conditions, thus preserving the immunoreactivity. In all commonly used buffers with pH ranging from 6.0 to 8.0, FabRICATOR digests human IgG efficiently (Table 2). Best activity is obtained at 37°C, but digestion can also be performed at room temperature with prolonged reaction time. Optimization is then required.

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

Immobilized FabRICATOR is available in convenient spin column format, FragIT[™], for generation of fragments without FabRICATOR in the final sample preparation.

Table 1. *FabRICATOR digestion site.*

IgG subclass	FabRICATOR digestion site
Human IgG1, IgG3, IgG4	..CPAPELLG / GPSVF..
Human IgG2	..CPAPPVA / GPSVF..

Unit Definition

One unit FabRICATOR digests $\geq 95\%$ of 1 μg human IgG when incubated in 10 mM sodium phosphate, 140 mM NaCl, 2.7 mM KCl, pH 7.4 at 37°C for 30 min.

Content and Storage

FabRICATOR is supplied lyophilized in 10 mM sodium phosphate, 140 mM NaCl, 2.7 mM KCl, pH 7.4, with no preservatives added.

FabRICATOR is shipped at ambient temperature and should be stored at -20°C upon arrival.

After reconstitution, FabRICATOR is stable for at least 1 month at +4-8°C.

FabRICATOR is for R&D use only.

This protocol is for FabRICATOR in vials.
For FabRICATOR in strips and plates, follow the protocol on page 8.

Additional Materials Required

- Digestion buffer: see Table 2^{1,2}.
- For FabRICATOR LE, use endotoxin-free materials and solutions.

Preparation of IgG

- Prepare IgG in a compatible digestion buffer, see Table 2^{1,2}. The final IgG concentration in the reaction should be 0.5-10 mg/ml.

Antibody Subunit Generation

1 Prepare FabRICATOR®

Reconstitute FabRICATOR according to Table 3⁴.

2 Add FabRICATOR®

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

3 Digestion

Incubate for 30 min at 37°C.

Table 2. Buffers and pH compatible with FabRICATOR digestion^{1,2}.

Compatible buffers	pH range
Phosphate buffer 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 ¹	

Table 3. Recommended volumes for reconstitution of FabRICATOR enzyme⁴.

Product	Product size	Reconstitution volume
A0-FR1-020	2000 units	30 µl ddH ₂ O
A0-FR1-050	5000 units	75 µl ddH ₂ O
A0-FR1-008	8 x 100 units	50-100 µl Ab / well ³
A0-FR1-096	96 x 100 units	50-100 µl Ab / well ³
A0-FR8-020	2000 units	30 µl ddH ₂ O (LE)
A0-FR8-050	5000 units	75 µl ddH ₂ O (LE)

Notes

1. FabRICATOR is compatible with several antibody formulation buffers.
2. FabRICATOR enzyme is irreversibly inactivated below pH 5.0. Digestion above pH 8.0 requires prolonged/optimized reaction conditions.
3. Reconstitution volume depends on antibody concentration, each well contains enough enzyme to digest 100 µg of antibody.
4. To prevent microbial contamination, sodium azide can be added to the solution to a final concentration of 0.02 - 0.05% (w/v).

Additional Materials Required

- Digestion buffer: see Table 2^{1,2}.

Preparation of IgG

- Prepare IgG in a compatible digestion buffer, see Table 2^{1,2}. The final IgG concentration in the reaction should be 1-2 mg/ml.

Antibody Subunit Generation

1 Add IgG

Add the IgG solution directly to the lyophilized FabRICATOR (50-100 μ l/well). Each well contains enough enzyme to digest 100 μ g of antibody. Penetrate the aluminium foil by using a standard pipette tip. Make sure to reconstitute all lyophilized material.

2 Digestion

Incubate for 30 min at 37°C.

Quality Control

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

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

Related Products

FabRICATOR® Z

Digestion of mouse IgG2a and IgG3

FragIT™

Immobilized FabRICATOR, digestion of IgG

FragIT™ kit

Digestion of IgG and purification of F(ab')₂ and Fc fragments

FabRICATOR®

Limited Use Label License: Research Use Only

The purchase of the **IdeS** enzyme from *Streptococcus pyogenes* (sold under the trade name FabRICATOR®) conveys to the purchaser the limited, non-transferable right to use the purchased amount of **IdeS** only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel.

Purchaser agrees to be bound by the following terms and restrictions:

1) A right is granted purchaser only for internal research purposes using **IdeS** for digesting an IgG and is not for use in commercial services of any kind, including, without limitation, reporting the result of purchaser's activities for a fee or other form of consideration.

2) **IdeS** will not be made available by purchaser to any third parties in any form, separately or in combination, for any monetary or other consideration or at no charge, except that **IdeS** may be made available to third parties who agree to be bound by all the terms and restrictions of this right for purposes of evaluation only.

3) **IdeS** and the digested IgG will not be used *in vivo* in humans.

4) Purchaser will not make commercial use of the **IdeS** unless it first

secures a Sublicense Agreement from Genovis AB for such commercial use.

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Genovis AB holds an exclusive world wide license to all patents derived from international publication WO03051914, including granted US Patent No US 7,666,582 regarding IdeS from *Streptococcus pyogenes* for biotechnical industrial applications which are neither therapeutic nor diagnostic, other than the following exception which is included within the license: digesting IgG *in vitro* in clinical samples for diagnostic purposes.

This license is defined in the intellectual property license agreement between Hansa Biopharma AB and Genovis AB.

The trademark FabRICATOR® is the property of Genovis AB.

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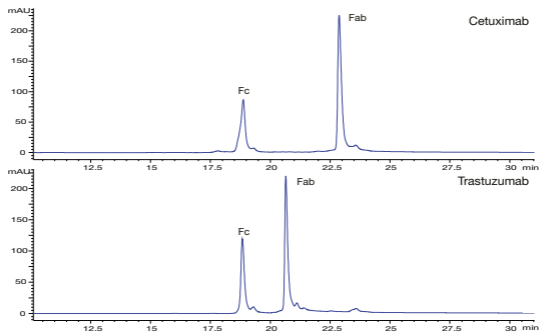
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FabALACTICA®

Generation of hIgG1 Fab Fragments – Digestion above the Hinge

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.





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