

INSTRUCTIONS

Version 15.1.1

Instructions for product no

A0-FR1-020	2000 units	Cleavage of up to 2.0mg IgG
A0-FR1-050	5000 units	Cleavage of up to 5.0mg IgG
A0-FR1-250	25000 units	Cleavage of up to 5 x 5.0 mg IgG
A0-FR4-060	3 x 2000u	Validation Kit

Content and Storage

FabRICATOR® is supplied as lyophilized powder with no preservatives added.

FabRICATOR® should be stored at -20°C upon arrival.

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

FabRICATOR® is for R&D use only.

Product Description

FabRICATOR® is an enzyme for preparation of F(ab')₂. FabRICATOR® is a digestive enzyme that cleaves IgG only at one specific site below the hinge region resulting in F(ab')₂ and Fc-fragments. Since FabRICATOR® only cleaves at one specific site below the hinge region, there is no risk of over digestion if the incubation time is increased for several hours.

FabRICATOR® cleaves all subclasses of human, monkey, rabbit and sheep IgG but only subclass IgG2a and IgG3 of mouse IgG. Cleavage of mouse IgG2a and IgG3 requires a special protocol with increased FabRICATOR® concentration and prolonged incubation time.

FabRICATOR® cleaves IgG under physiological reaction conditions thus preserving the immunoreactivity. In all commonly used buffers with pH ranging from 6.0 to 8.0, FabRICATOR® cleaves human IgG efficiently (Table 1). Best activity is obtained at 37°C but digestion can also be performed at room temperature using prolonged reaction time. Optimization is required.

FabRICATOR® contains a His-tag and can therefore easily be removed after digestion.

Unit Definition

One unit cleaves ≥ 95% of 1µg human IgG when incubated in 10 mM sodium phosphate, 137 mM NaCl, 2.7mM KCl pH 7.4 at 37°C for 30min.

Quality Control

FabRICATOR® is tested to ensure lot-to-lot consistency.

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

Additional Materials Required

- Cleavage Buffer: see Table 1
- Test tubes

Table 1. Buffers tested for compatibility with FabRICATOR digestion at different pH.

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

Note! FabRICATOR® enzyme is irreversibly inactivated below pH 5.0. Digestion above pH 8.0 requires prolonged/optimized reaction conditions.

Method

Sub-unit fragmentation of IgG

- Make sure your antibody is in cleavage buffer (Table 1) with a concentration of 0.5-10mg/ml.
- Reconstitute FabRICATOR® in double distilled H₂O according to Table 2 below. To prevent microbial contamination, sodium azide can be added to the solution to a final concentration of 0.02 - 0.05% (w/v).
- Add FabRICATOR® to the IgG. Add **1 unit FabRICATOR®/1µg IgG**. *For cleavage of mouse IgG2a and IgG3 a special protocol is required, see below.
- Incubate for **30 minutes** in cleavage buffer at **37°C**. *For cleavage of mouse IgG2a and IgG3 a special protocol is required, see below.

Table 2.Volumes for reconstitution of FabRICATOR® enzymes.

Product	Product Size	Reconstitution volume	Concentration	Volume for 1000 units
A0-FR1-020	2000 units	30µL	67 units/µL	15µL
A0-FR1-050	5000 units	75µL	67 units/µL	15µL

*Cleavage of mouse IgG2a and IgG3

- Make sure your antibody is in cleavage buffer (Table 1) with a concentration of 0.5-10mg/ml.
- Reconstitute FabRICATOR® in double distilled H₂O according to Table 2 above. To prevent microbial contamination, sodium azide can be added to the solution to a final concentration of 0.02 - 0.05% (w/v).
- Add FabRICATOR® to the mouse IgG. Add **5 units FabRICATOR®/1µg mouse IgG**.
- Incubate for **2-3 hours** in cleavage buffer at **37°C**. Optimization is required for your specific antibody.

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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