

FabULOUS®

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-20°C



SmartEnzymes™



GENOVIS

INSTRUCTIONS FOR PRODUCT

FabULOUS[®] 2000 units (A0-PU1-020)

Digestion of up to 2 mg IgG

1 Prepare FabU LOUS®

Reconstitute FabU LOUS in 50 μl ddH₂O to a concentration of 40 units/ μl .

**2 Add FabU LOUS®**

Add 1 unit FabU LOUS / 1 μg IgG. Add reducing agent according to Table 2.

**3 Digestion**

Incubate for 1 h at 37°C.



PRODUCT DESCRIPTION

FabULOUS (SpeB) is an enzyme for digestion of IgG in the hinge region. Human IgG and IgG from mouse, rat, goat, sheep and rabbit are digested by FabULOUS, yielding Fab and Fc fragments¹. FabULOUS is active on IgG under reducing conditions. Strong reducing conditions during the reaction is likely to reduce interchain thiols. Using milder reducing conditions (i.e. 50 mM cysteine), FabULOUS will generate intact Fab fragments. The primary digestion site on human IgG1 is ..KTHT / CPPCPAPE..

A digestion protocol at 37°C for 1 h under reducing conditions is generally applicable³. FabULOUS digests IgG in commonly used buffers with pH ranging from 6.5 to 8.0 (Table 1). Optimal activity is obtained at 37°C.

FabULOUS is derived from *Streptococcus pyogenes* and expressed in *E. coli*. The enzyme contains a His-tag and the molecular weight is 29 kDa.

Unit Definition

One unit FabULOUDS digests $\geq 95\%$ of 1 μg human IgG1 in PBS, 5 mM DTT or TCEP, pH 7.4 at 37°C for 1 h.

Content and Storage

FabULOUDS is supplied lyophilized in 10 mM Tris, 150 mM NaCl, pH 7.6, with no preservatives added.

FabULOUDS is shipped cold and should be stored at -20°C upon arrival.

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

FabULOUDS is for R&D use only.

Additional Materials Required

- Digestion buffer: see Table 1.
- Reducing agent: see Table 2.

Preparation of IgG

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

Antibody Subunit Generation

1 Prepare FabULOUS®

Reconstitute FabULOUS in 50 μ l ddH₂O to a concentration of 40 units/ μ l.

2 Add FabULOUS®

- Add 1 unit FabULOUS / 1 μ g IgG.
- Add reducing agent to the digestion reaction to a final concentration of 1-5 mM DTT or 1-5 mM TCEP. See Table 2 for other compatible reducing agents.

3 Digestion

Incubate for 1 h at 37°C.

Table 1. Buffers and pH compatible with FabULOUS digestion.

Compatible buffers	pH range
Phosphate buffered saline (PBS)	6.5 – 8.0
Tris buffer	7.0 – 8.0
Sodium acetate buffer	6.5 ²

Table 2. Reducing agents compatible with FabULOUS.

Reducing agent	Concentration (mM)	pH
DTT	1-5	6.5 - 8.0
TCEP	1-5	6.5 - 8.0
Cysteine	50-100	6.5 - 8.0
Cysteamine	50-100	6.5 - 8.0
2-Mercaptoethanol	50-100	6.5 - 8.0

Notes

1. Fab' is generated from human IgG4.
2. Below pH 6.5, the enzyme concentration needs to be increased for efficient digestion of IgG.
3. For digestion of human IgG4, the incubation time has to be increased. Optimization of incubation time (up to overnight) and reducing conditions are needed.

Quality Control

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

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

Related Products

FabALACTICA®

Generation of Fab fragments from hIgG1

FabULOUS® Fab kit

Generation and purification of Fab fragments from mouse IgG

FabULOUS®

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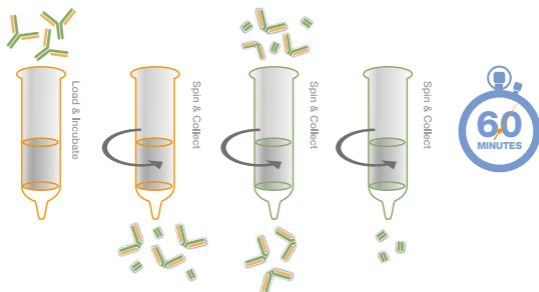
Genovis products are intended for research use only. They are not intended to be used for therapeutic or diagnostic purposes in humans or animals.

FragIT™ kit

Digestion of IgG and Purification of F(ab')₂ and Fc Fragments

FragIT kit consists of an IgG digestion column, FragIT, and an affinity purification column, CaptureSelect™*. FragIT is a resin with FabRICATOR® enzyme covalently coupled to agarose beads for digestion of IgG to generate F(ab')₂ 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')₂ and Fc fragments.



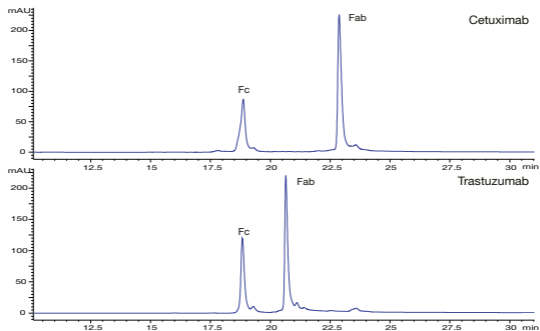
**Made with Thermo Scientific™ CaptureSelect™ resin from Thermo Fisher Scientific Inc. and its subsidiaries. Thermo Scientific and CaptureSelect are trademarks of Thermo Fisher Scientific Inc. and its subsidiaries.*

FabALACTICA®

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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