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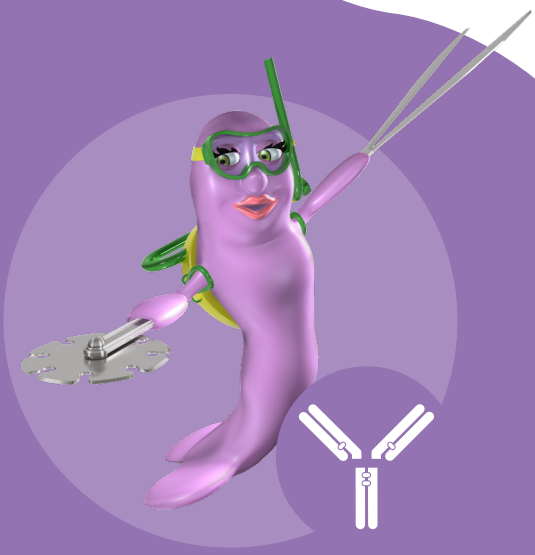

FabULOUS™

Lyophilized

STORE AT
-20°C

FOR RESEARCH USE ONLY

Instructions for Use

FabULOUS™ Lyophilized 2000 units (A0-PU1-020)
Process 2 mg IgG

DOWNLOAD INSTRUCTIONS FOR USE
www.genovis.com/ifu-A0-PU1

Lyophilized Enzyme for Above Hinge Digestion of IgG

FabULOUS (SpeB) is a cysteine protease that digests in the hinge region of IgG from several different species and subclasses, generating Fab and Fc fragments. Human, mouse, rat, goat and sheep IgG are digested by FabULOUS.[1]

FabULOUS is active on IgG under reducing conditions. Strong reducing conditions during the reaction is likely to reduce interchain disulfide bonds. For IgG from some species, FabULOUS is active also under mild reducing conditions that allow for the generation of intact Fab fragments with no reduction of interchain disulfide bonds. Intact Fab fragments can also be achieved if a buffer exchange is performed after digestion at stronger reducing conditions. This enables reformation of the disulfide bonds in the Fab.

The primary digestion site on human IgG1 is …KTHT / CPPCPAPE… A digestion protocol at 37°C for 1 hour under reducing conditions is generally applicable.[2] FabULOUS digests IgG in commonly used buffers with pH 6.5-8.0 (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 FabULOUS Lyophilized digests ≥ 95% of 1 µg human IgG1 when incubated in PBS (10 mM sodium phosphate, 140 mM NaCl, 2.7 mM KCl), pH 7.4, with 5 mM DTT or TCEP at 37°C for 1 hour.

CONTENT AND STORAGE

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

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

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

FabULOUS is for R&D use only.

QUALITY CONTROL

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

FabULOUS 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

FabULOUS™ Fab Kit
Lyophilized enzyme and affinity resin for above hinge digestion of murine IgG and purification of fragments

FabALACTICA™
Above hinge digestion of human IgG1

1. Fab' fragments are generated from human IgG4.
2. For digestion of human IgG4, the incubation time has to be increased. Optimization of incubation time (up to overnight) and reducing conditions are needed.

Preparations

Additional Materials Required

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

Sample Preparation

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

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[3]

Table 2. Reducing Agents Compatible with FabULOUS

Reducing Agent	Final Concentration (mM)	pH
DTT	1-5	6.5 - 8.0
TCEP	1-5	6.5 - 8.0
L-cysteine	50-100	6.5 - 8.0
L-cysteamine	50-100	6.5 - 8.0
2-Mercaptoethanol	50-100	6.5 - 8.0

3. At pH < 6.5, the enzyme concentration needs to be increased for efficient digestion of IgG.

Above Hinge Digestion of IgG

1. Prepare FabULOUS

1.1 Reconstitute FabULOUS in 50 µl ddH_2O to a concentration of 40 units/µl.

2. Add FabULOUS

2.1 Add 1 unit FabULOUS / 1 µg IgG.

2.2 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. Enzymatic Reaction

3.1 Incubate for 1 h at 37°C.

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