

## INSTRUCTIONS

Version 17.1.1

Instructions for product no: A0-AG1-020                      2000 units                      Digestion of up to 2 mg hIgG1

### Content and Storage

FabALACTICA™ is supplied lyophilized in 10 mM sodium phosphate, 137 mM NaCl, 2.7 mM KCl pH 7.4, with no preservatives added.

FabALACTICA™ is shipped on ice and should be stored at -20 °C upon arrival.

After reconstitution FabALACTICA™ is stable for 1 month at +4-8 °C.

FabALACTICA™ is for R&D use only.

### Product Description

FabALACTICA™ (IgdE) is an enzyme that digests human IgG1 at one specific site just above the hinge. No reducing agent is required for enzymatic activity thus intact Fab and Fc fragments are generated. The FabALACTICA™ enzyme digestion site on human IgG1 is ..KSCDKT / HTCPCP.. There is no risk of overdigestion with prolonged digestion time.

FabALACTICA™ digests hIgG1 under physiological reaction conditions thus preserving the immunoreactivity. Optimal activity is obtained at pH 6.5 to 7.5 and at 37 °C. Digestion can also be performed at room temperature with slightly lower yield than at 37 °C.

FabALACTICA™ is derived from *Streptococcus agalactiae* and expressed in *E. coli*. The enzyme contains a His-tag and the molecular weight is 70 019 Da.

### Unit Definition

One unit digests ≥ 90 % of 1 µg human IgG1 when incubated in 150 mM sodium phosphate, pH 7.0 at 37 °C overnight (16-18 h).

### Quality Control

FabALACTICA™ is tested to meet specification.

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

### Protocol

#### Additional Materials Required

Digestion buffer<sup>1</sup>: 150 mM sodium phosphate, pH 7.0.

#### Preparation of IgG

Prepare the IgG (human IgG1) in digestion buffer. The final IgG concentration in digestion reaction should be 0.5-10 mg/ml.

#### Antibody Subunit Generation

1. Reconstitute FabALACTICA™ in 50 µl ddH<sub>2</sub>O<sup>2</sup> to a concentration of 40 units / µl.
2. Add FabALACTICA™ to the IgG<sup>3</sup>. Add **1 unit FabALACTICA™ / 1 µg hIgG1**.
3. Incubate at **37 °C overnight (16-18 h)**<sup>4</sup>.

#### Notes

1. Optimal activity is obtained in 100-150 mM sodium phosphate buffers at pH 6.5-7.5. Sodium chloride up to 150 mM can be added without affecting the enzyme activity.
2. To prevent microbial contamination, sodium azide can be added to the solution to a final concentration of 0.02 - 0.05% (w/v).
3. A higher enzyme concentration may increase digestion efficiency of individual antibodies. This requires optimization.
4. Shorter incubation times (i. e. 3-6 hours) may be used if a lower digestion yield is acceptable.

## Reference

Spoerry C, Hesse P, Lewis MJ, Paton L, Woof JM, von Pawel-Rammingen U (2016) Novel IgG-Degrading Enzymes of the IgdE Protease Family Link Substrate Specificity to Host Tropism of Streptococcus Species. PLoS ONE 11(10): e0164809. doi:10.1371/journal.pone.0164809

### **FabALACTICA™**

**Limited Use Label License: Research Use Only**

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All rights reserved. Aspects of FabALACTICA™ technology are encompassed by pending patent applications, Swedish patent application (SE1630021-2) and a PCT application (PCT/EP2017/052463), in the name of Genovis AB.

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