FabALACTICA®

FOR RESEARCH USE ONLY

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SmartEnzymes™



FabALACTICA[®]

INSTRUCTIONS FOR PRODUCT

FabALACTICA[®] 2000 units (A0-AG1-020) Digestion of up to 2 mg human IgG1

Last revised Nov 2019

QUICK GUIDE



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



Add 1 unit FabALACTICA / 1 µg human IgG1







FabALACTICA (IgdE) is an enzyme that digests human IgG1 at a specific site above the hinge, generating intact Fab and Fc fragments (1). No reducing agent is required for enzymatic activity. The digestion site on human IgG1 is ..KSCDKT / HTCPPCP. There is no risk of overdigestion if the incubation time is prolonged.

Since FabALACTICA digests hlgG1 under physiological reaction conditions, the immunoreactivity is preserved. 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 kDa.

Unit Definition

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

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

After reconstitution, the FabALACTICA enzyme is stable for at least 1 month at $+4-8^{\circ}$ C.

FabALACTICA is for R&D use only.

DETAILED PROTOCOL

Additional Materials Required

• Digestion buffer¹: 150 mM sodium phosphate, pH 7.0.

Sample Preparation

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

Reconstitute FabALACTICA in 50 μ I ddH₂O to 40 units/ μ I².

Add FabALACTICA®

Add 1 unit FabALACTICA / 1 µg human IgG13.



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Digestion

Incubate overnight (16-18 h) at 37°C4.

Notes

- 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.
- To prevent microbial contamination, sodium azide can be added to the solution to a final concentration of 0.02 - 0.05% (w/v).
- A higher enzyme concentration may increase digestion efficiency of individual antibodies. This requires optimization.
- Shorter incubation times (i. e. 3-6 hours) may be used if a lower digestion yield is acceptable.

Quality Control

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

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

Product Reference

1. Spoerry, C. et al., 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.

Related Products

Immobilized FabALACTICA®

Immobilized FabALACTICA enzyme, digestion of human IgG1

FabALACTICA® Fab kit

Generation and purification of intact Fab fragments from human IgG1

FabRICATOR®

Digestion of IgG below the hinge

FabALACTICA®

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GlyCLICK[®]

Site-specific Labeling of Antibodies

GlyCLICK is a site-specific conjugation technology for antibodies based on enzymatic remodeling of the N-linked Fc-glycans and click chemistry^{*}.

- Degree of label (DOL) = 2
- Intact immunoreactivity
- A variety of labels can be conjugated to the antibody, including drugs, chelators, biotin and fluorophores.



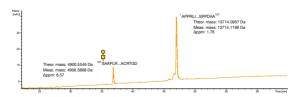
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OpeRATOR[®]

O-glycan-specific Endoprotease

OpeRATOR is a novel tool for analysis of mucintype O-glycans on glycoproteins. The protein binds to O-glycans and digests the peptide backbone N-terminally of the S/T glycosylation sites.

- O-glycan-specific, mucin-type
- Requires O-glycans for activity
- Generates glycopeptides with O-glycans and allows for O-glycan profiling and site occupancy determination using mass spectrometry.



Erythropoletin (EPO) is a ~30 kDa glycoprotein with one core 1 O-glycan site. The protein was used here as a substrate to demonstrate the specific activity of the OpeRATOR protease. OpeRATOR hydrolyzed the protein N- terminally of the serine O-glycan site, and after reduction of dsulfide bridges, the resulting two fragments were separated and intact mass was analyzed by Q-TOF MS using ESI.



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