FabDELLO[™]

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STORE CONTENT AT DIFFERENT TEMPERATURES (See page 5)



SmartEnzymes™



INSTRUCTIONS FOR PRODUCTS

FabDELLO 8×100 units (B1-BD1-008) Digestion of up to 8×100 µg human lgG1 (8 well strip)

FabDELLO 96 ×100 units (B1-BD1-096) Digestion of up to 96 ×100 µg human IgG1 (96 well plate)

Quick Guide

- The Quick Guide (p. 3) is intended for experienced users. First time users are recommended to follow the detailed protocol (p. 6).
- Cover the enzyme vial with adhesive plastic or move the reaction to a new vial during the incubation.

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WORKFLOW

Quick Guide



• Add 100 µg IgG to the enzyme vial.



- Add TBS buffer to a total volume of 95 µl.
- Add 5 µl 20× CaCl₂ solution (200 mM).





• Incubate for 2h at 37°C.



FabDELLO is an enzyme that digests human IgG1, yielding intact Fab and Fc fragments. Under native conditions, FabDELLO digests human IgG1 at one single site in the upper hinge (..KSCDK / THTCPPCP..). A second digestion site on the Fc may appear if the N-glycans are removed. FabDELLO requires the presence of calcium ions to be active. Optimal activity is obtained at 37°C and pH 7-8.5.

FabDELLO is cloned from *Bdellovibrio bacteriovorus* and expressed in *E. coli*. The enzyme contains a His-tag and the molecular weight is 32 kDa.

Unit Definition

One unit FabDELLO digests≥90% of 1µg human IgG1, when incubated in Tris buffered saline (TBS), pH 7.6, containing 10mM CaCl₂, at 37°C for 2 h.

Content and Storage

FabDELLO is supplied lyophilized in 50 mM Tris-HCl, 150 mM NaCl, pH 7.6, with no preservatives added. The enzyme is supplied together with 1 vial $20 \times CaCl_2$ solution (200 mM). FabDELLO is shipped cold and the FabDELLO enzyme should be stored at -20°C upon arrival. The CaCl_2 solution should be stored at +4-8°C upon arrival. FabDELLO has autoproteolytic activity. Therefore, the enzyme should be kept cold and used within the day of reconstitution.

Additional Materials Required

• Digestion buffer: TBS (50 mM Tris-HCl, 150 mM NaCl), pH 7.6.

DETAILED PROTOCOL

Sample Preparation

Prepare the human IgG1 in the digestion buffer. The final IgG concentration in the digestion reaction should be 0.5-5 mg/ml.

1 Add IgG

- Pierce the aluminium foil with a pipet tip.
- Add 100µg IgG to the enzyme vial¹.

2 Add Buffer

Add TBS buffer to a total volume of 95 µl^{2,3}.

3 Add CaCl₂

Add 5µl 20× CaCl₂ solution (200 mM)⁴.

4 Digestion

- Mix the solution by aspirating and dispensing the liquid a few times.
- Cover the vial with adhesive plastic or move the content to another vial.
- Incubate for 2h at 37°C⁵.

FabDELLO[™]

Quality Control

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

Related Products

FabALACTICA®

Above hinge digestion of human IgG1

Notes

- The enzyme may also be dissolved in ultrapure water and added to a digestion in another vial if digestion of smaller amounts of IgG is desired.
- Optimal activity is achieved in TBS buffer pH 7-8.5. Buffers containing phosphate should be avoided since the phosphate ions will form the insoluble calcium phosphate salt together with the calcium ions required for enzymatic activity.
- 3. Another buffer volume may be added. The total volume in the enzyme vial should be 20-200 µl.
- The final CaCl₂ concentration should be 10mM. If the reaction volume is adjusted, the volume of the CaCl₂ solution must also be adjusted.
- 5. The digestion time may need to be optimized for individual antibodies.

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