FOR RESEARCH

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SmartEnzymes™



INSTRUCTIONS FOR PRODUCTS

FabRICATOR MagIC 2 ml (A0-FRM-024) Digestion of up to 24 x 200 µg IgG

FabRICATOR MagIC 4 x 2 ml (A0-FRM-096) Digestion of up to 96 x 200 μg IgG

Digestion - FabRICATOR MagIC

- 1 Resuspend the FabRICATOR MagIC resin by careful inversion of the vial until a homogenous slurry is observed.
- 2 Dispense 80 µl FabRICATOR MagIC slurry per well into the desired number of wells of a 96-well plate or into microtubes.
- Wash FabRICATOR MagIC resin in PBS by magnetic separation.
- 4 Mix FabRICATOR MagIC resin with 100 μl of IgG sample with the concentration of 0.2-2.0 mg/ml.
- 5 Incubate 10-20 min at 37°C with appropriate mixing.
- 6 Remove FabRICATOR MagIC resin by magnetic separation.

PRODUCT DESCRIPTION

FabRICATOR MagIC is a resin with the FabRICATOR enzyme covalently coupled to magnetic agarose beads for IgG subunit generation. After fragmentation, the FabRICATOR MagIC resin is easily removed by magnetic separation and pure F(ab')2 and Fc fragments are quickly recovered without the presence of FabRICATOR in the final preparation.

FabRICATOR MagIC can:

- digest all subclasses of human IgG as well as some classes of monkey, rat, rabbit and sheep IgG.
- digest IgG at a specific site below the hinge region, and there is no risk of overdigestion if the incubation time is prolonged.
- be used with all commonly used buffers with a pH from 6.0 to 8.0. Optimization may be required.

Content and Storage

Each vial of FabRICATOR MagIC contains sufficient material to digest up to 24 x 200 µg IgG. The resin is supplied in 20% EtOH with no preservatives added.

FabRICATOR MagIC is shipped cold and should be stored at +4-8°C upon arrival. **Do not freeze the product!**

FabRICATOR MagIC is for R&D use only.

DETAILED PROTOCOL

Additional Materials Required

 Digestion buffer¹: 10 mM Sodium Phosphate, 150 mM NaCl, pH 7.4 (PBS).

Sample Preparation

- Prepare the antibody in the digestion buffer¹ at a concentration of 0.2-2.0 mg/ml.
- It is possible to add 0.05% Polysorbate 20 to the digestion buffer to minimize shear stress.
- For generation of reduced subunits, Fd', LC and scFc, add TCEP to a final concentration of 5 mM to the sample.

Digestion of IgG using FabRICATOR MagIC

FabRICATOR MagIC can either be used in automated systems designed to handle magnetic beads or in manual sample preparation with a 96-well plate, a plate shaker and a 96-well magnetic plate for magnetic bead separation.

FabRICATOR MagIC resin needs to be washed with PBS prior to mixing it with the sample.

Settled resin from 80 µl FabRICATOR MagIC can digest 20-200 µg lgG in a 100 µl sample volume when incubated 20 min² at 37°C.

Resuspend

 Resuspend the FabRICATOR MagIC resin by careful inversion of the vial until a homogenous slurry is observed.

2 Dispense

- Dispense 80 µl of the FabRICATOR MagIC slurry per well into the desired number of wells of a 96-well plate (or microtubes). It is important to invert the vial between each dispensing to keep the resin resuspended.
- For automated systems* it is beneficial to use a 96-deepwell plate which can be transferred directly to the autosampler of the LC-MS or other analytical platform for the subsequent analysis.

3 Wash

 Wash the FabRICATOR MagIC resin in PBS by magnetic separation. It is possible to wash once in a larger volume, for example 1000 μl, or several times in smaller volumes, for example 3 x 200 μl.

DETAILED PROTOCOL

4 Add FabRICATOR MagIC

 Mix the FabRICATOR MagIC resin with 100 µl of IgG sample with the concentration of 0.2-2.0 mg/ml.

5 Digestion

 Incubate 10-20 min at 37°C with adequate mixing to avoid sedimentation of the magnetic resin, ensuring equal distribution of the resin in the sample solution during the incubation.

6 Remove FabRICATOR MagIC

 Remove the FabRICATOR MagIC resin by magnetic separation.

^{*} More detailed instructions for a workflow using the Thermo Scientific™ KingFisher™ instrument and protocol for the BindIt™ software are available at www.genovis.com

Notes

- 1. Other commonly used buffers at physiological pH and ionic strength can also be used. Optimization may be required.
- 2. Incubation time can be increased without the risk of overdigestion of the antibody.

Quality Control

FabRICATOR MagIC is tested to meet the specifications and lot-to-lot consistency.

FabRICATOR MagIC is tested for the absence of microbial contamination using blood agar plates, Sabouraud dextrose agar plates and fluid thioglycollate medium.

Related Products

FragIT

Immobilized FabRICATOR, digestion of IgG

FabRICATOR-HPLC

FabRICATOR

Digestion of IgG below the hinge

Limited Use Label License: Research Use Only

Limited Use Label License: For research use only. Not intended for any animal or human therapeutic or diagnostic use.

The purchase of the IdeS enzyme from Streptococcus pyogenes (sold under the trade name FabRICATOR®) conveys to the purchaser the limited, non-transferable right to use the purchased amount of IdeS only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel.

Purchaser agrees to be bound by the following terms and restrictions:

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- 2) IdeS will not be made available by purchaser to any third parties in any form, separately or in combination, for any monetary or other consideration or at no charge, except that IdeS may be made available to third parties who agree to be bound by all the terms and restrictions of this right for purposes of evaluation only.
- 3) IdeS and the digested IgG will not be used in vivo in humans.
- 4) Purchaser will not make commercial use of the IdeS enzyme unless it first secures a Sublicense Agreement from Genovis AB for such commercial use.

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