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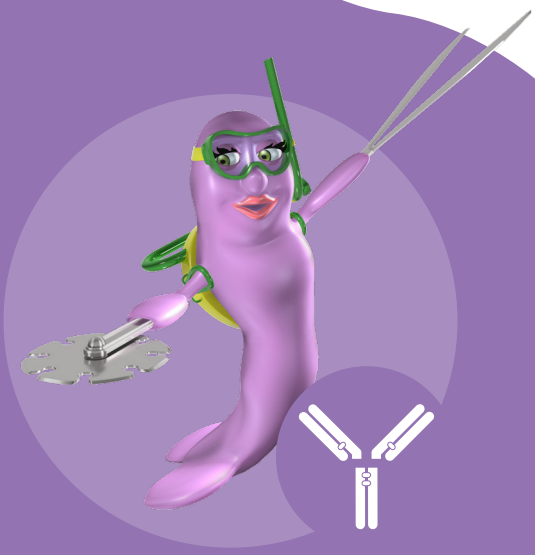

FabULOUS™

Fab Kit

STORE AT
DIFFERENT
TEMPERATURES

FOR RESEARCH USE ONLY

Instructions for Use

FabULOUS™ Fab Kit 2 mg (A1-PFK-020)
Process 2 mg mouse IgG

DOWNLOAD INSTRUCTIONS FOR USE
www.genovis.com/ifu-A1-PFK

Lyophilized Enzyme and Affinity Resin for Hinge-region Digestion of Murine IgG and Purification of Fragments

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.

FabULOUS Fab Kit consists of FabULOUS Lyophilized for digestion of IgG, and spin columns with CaptureSelect™* LC-kappa (mur) resin for affinity purification of murine Fab fragments.[1] FabULOUS digests 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.

FabULOUS is derived from *Streptococcus pyogenes* and expressed in *E. coli*. The enzyme contains a His-tag and has a molecular weight of 29 kDa.

The ligand on the CaptureSelect™ LC-kappa affinity resin is directed to a unique domain of the constant part of the kappa light chain of murine IgG, which enables binding and purification of intact Fab fragments. If the sample is mouse IgG with LC-lambda, please contact the Genovis team. The CaptureSelect LC-lambda (mouse) is available upon request.

CONTENT AND STORAGE

FabULOUS Fab Kit contains two components. The product box is shipped cold, and the two components should be stored at different temperatures upon arrival.

- **1 vial FabULOUS Lyophilized** is supplied lyophilized in 50 mM Tris, 138 mM NaCl, 2.7 mM KCl, pH 7.6, with no preservatives added. One unit FabULOUS Lyophilized digests ≥ 95% of 1 µg of human IgG1 in PBS pH 7.4 with 5 mM DTT or TCEP at 37˚C for 1 hour. This is also valid for 1 µg of mouse IgG1 in PBS with 30-50 mM L-cysteine as reducing agent. FabULOUS Lyophilized should be stored at -20°C upon arrival. After reconstitution, FabULOUS Lyophilized is stable for at least 1 month at +4-8°C.
- **4 CaptureSelect™ LC-kappa (mur) Microspin columns** are supplied in 20% ethanol with no preservatives added. One column contains sufficient material to purify 0.5 mg IgG. The columns should be stored at +4-8°C upon arrival. **Do not freeze the columns!**

FabULOUS Fab Kit is for R&D use only.

* Made with Thermo Scientific™ CaptureSelect™ resin from Thermo Fisher Scientific Inc. and its subsidiaries. Thermo Scientific and CaptureSelect are trademarks of Thermo Fisher Scientific Inc. and its subsidiaries.

1. The FabULOUS Fab Kit is developed for mouse IgG. Rat IgG might also be processed, but the digestion may need some optimization.

QUALITY CONTROL

FabULOUS Lyophilized included in FabULOUS Fab Kit 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™ Lyophilized
Lyophilized enzyme for hinge-region digestion of IgG

FabRICATOR® Fab2 Kit
Immobilized enzyme and affinity resin for below hinge digestion of IgG and purification of fragments

FabALACTICA™ Lyophilized
Lyophilized enzyme for above hinge digestion of human IgG1

FabALACTICA™ Fab Kit
Immobilized enzyme and affinity resin for above hinge digestion of human IgG1 and purification of Fab fragments

Preparations

Important Information

- Prepare a stock solution of 1 M L-cysteine in ddH_2O (90 µl aliquots may be stored at -20°C).
- To neutralize the L-cysteine solution, thaw one vial and add 10 µl 8 M NaOH to the 90 µl L-cysteine solution. This gives 100 µl of 0.9 M cysteine solution at neutral pH ready to be used.
- The L-cysteine solution at neutral pH needs to be freshly prepared and used the same day. Use the prepared solution within 6 hours!

Additional Materials Required

- Reaction buffer: see Table 1.
- Binding buffer: PBS or TBS, pH 7.0-7.5 (physiological pH and ionic strength).
- 0.9 M L-cysteine solution at neutral pH.
- Elution buffer: 0.1 M glycine, pH 2.5.
- Neutralizing buffer: 1 M Tris, pH 8.0.
- Microcentrifuge tubes: 1.5 and 2 ml.

Table 1. Buffers Tested for Compatibility with FabULOUS Fab Kit Digestion

Compatible Buffers	pH Range
Phosphate-buffered saline (PBS)	6.5-8.0
Tris-buffered saline (TBS)	7.0-8.0

Hinge-region Digestion of Murine IgG

Sample Preparation
Prepare the mouse IgG in the reaction buffer. The final IgG concentration should be in the range of 1-10 mg/ml. Digest from 0.5 mg mouse antibody in 100-400 µl can be purified on one CaptureSelect™ LC-kappa (mur) column.

1. Prepare FabULOUS

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

2. Add FabULOUS

2.1 Add 1 unit FabULOUS / 1 µg IgG.

2.2 Add L-cysteine solution to the reaction to a final concentration of 30-50 mM.

3. Enzymatic Reaction

3.1 Incubate for 1 h[2] at 37°C.

2. Digestion time may need to be optimized for individual antibodies.

Purification of Fab Fragments

Important Information

- Each CaptureSelect™ LC-kappa (mur) spin column can purify Fab from 0.5 mg mouse IgG.
- Use lids and bottom caps during the incubation.
- Before centrifugation, remove the bottom cap and loosen the lid (do not remove the lid).

4. Equilibration

4.1 Break off the bottom cap of the CaptureSelect™ column[3] (save the cap) and place the column in a microcentrifuge tube. Loosen the lid.

4.2 Centrifuge at 1000 × g for 1 min to remove the storage solution. Discard the flow-through.

4.3 Equilibrate the column by adding 300 µl binding buffer and centrifuge at 1000 × g for 1 min. Discard the flow-through.

4.4 Perform step 4.3 two additional times.

4.5 Insert the bottom cap.

5. Binding of Fab Fragments

5.1 Add the digested sample from step 3.1 to the CaptureSelect™ column and seal the column with the lid. Up to 0.5 mg of digested IgG in a volume of 100-400 µl can be added to each column. **Note!** Minimum volume added to each column should be 100 µl, to ensure proper mixing with the resin.

5.2 Fully suspend the media, mix by inversion and make sure there is a flow in the column.

5.3 Incubate the column with end-over-end mixing at room temperature for 30-60 min.

6. Collection of Fc Fragments

6.1 Remove the bottom cap and place the column in a new 2 ml microcentrifuge tube. Loosen the lid.

6.2 Centrifuge at 1000 × g for 1 min to collect the Fc fragments[4].

3. Four CaptureSelect LC-kappa (mur) Microspin columns are included. Each column contains sufficient material to purify 0.5 mg IgG.
4. Note that the FabULOUS enzyme is also present in this sample.

Elution of Fab Fragments

7. Wash

7.1 Place the column from step 6.2 in a new microcentrifuge tube and add 300 µl binding buffer.

7.2 Centrifuge at 1000 × g for 1 min. Discard the flow-through.

7.3 Perform steps 7.1-7.2 two additional times.

8. Elution of Fab Fragments

8.1 Insert the bottom cap.

8.2 Prepare a 2 ml microcentrifuge tube with 80 µl neutralizing buffer (0.2 × the elution volume).

8.3 Add 400 µl elution buffer and seal the column with the lid.

8.4 Fully suspend the media by manually inverting the column a couple of times.

8.5 Remove the bottom cap and place the column in the prepared microcentrifuge tube. Loosen the lid.

8.6 Centrifuge at 1000 × g for 1 min to elute the Fab fragments.

8.7 Perform steps 8.1-8.6 two additional times for maximum recovery.

8.8 Pool the eluted Fab fractions and make sure that the pH is neutralized.

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