FabULOUS® Fab kit

GENOVIS

INSTRUCTIONS

Version 16.1.1

Instructions for Product
FabULOUS® Fab kit mouse (A1-PFK-020) Digestion and purification of up to 2mg mouse IgG

Product Description

FabULOUS® Fab kit mouse is used for rapid and easy purification of Fab fragments from mouse IgG antibodies.
The FabULOUS® (SpeB) enzyme digests IgG from many species, including mouse, in the hinge region. The FabULOUS® Fab kit mouse is designed for digestion and purification of Fab fragments from mouse IgG antibodies. FabULOUS® is only active at reduced reaction conditions and incubation at optimized reducing conditions generates intact Fab fragments from mouse IgG antibodies. The FabULOUS® Fab kit mouse consists of 2000 units of the FabULOUS® enzyme for digestion of 2 mg of IgG. Included in the kit are CaptureSelect™LC-kappa[1] (mur) affinity purification spin columns for easy purification of the prepared Fab fragments from mouse IgG[2].

FabULOUS® digests IgG in commonly used buffers, with pH ranging from 6.5 to 8.0 (Table 1). Optimal activity is obtained at 37°C. FabULOUS® requires reduced conditions in the digestion reaction. A concentration of 30-50 mM cysteine is enough to obtain enzymatic activity and it should not be exceeded when intact Fab fragments are required. Use of the reducing agents DTT and TCEP will not yield intact Fab fragments.

The CaptureSelect™ LC-kappa Affinity Matrix enables purification of mouse Fab fragments. The ligand is directed towards a unique domain on the constant part of the kappa light chain of murine immunoglobulins, which makes the CaptureSelect™ LC-kappa Affinity Matrix a unique resin in terms of specificity.

Unit Definition

1 unit of FabULOUS® digests 1 µg IgG.

Content and Storage

- 1x FabULOUS® 2000u, supplied as a lyophilized powder formulated in Tris/NaCl. One unit of FabULOUS® enzyme digests ≥ 95% of 1µg mouse IgG1 when incubated in physiological buffer at pH 6.5-8.0 at 37°C for 1h.
- 4x CaptureSelect™ LC-kappa (mur) MicroSpin columns, one column includes sufficient material to purify up to 0.5 mg mouse IgG. It is supplied in 20% EtOH.

FabULOUS® Fab kit mouse is shipped on ice.
FabULOUS® 2000u should be stored at -20°C upon arrival.
CaptureSelect™ LC-kappa resin in MicroSpin columns should be stored at +4-8°C.
After reconstitution of FabULOUS® enzyme, it is stable for 2 months at +4-8°C.
FabULOUS® Fab kit mouse is for R&D use only.

[1] 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.
[2] It is also possible to purify lambda light chain by using CaptureSelect LC-lambda, which is available upon request.

Additional Materials Required

- Digestion Buffer: See table 1
- Binding buffer: PBS or TBS, pH 7.0-7.5 (physiological pH and ionic strength)
- L-Cysteine solution pH neutral
- Elution buffer: 0.1 M Glycine, pH 2.5
- Neutralizing buffer: 1 M Tris, pH 8.0
- Reaction/Collection tubes: Micro centrifuge tubes (1.5-2 ml)

Table 1. Buffers tested for compatibility with FabULOUS® Fab kit digestion at different pH.

Compatible buffers	pH range
Phosphate buffered saline (PBS)	6.5 – 8.0
Tris buffered saline (TBS)	7.0 – 8.0

Protocol

Preparation of cysteine

- Prepare cysteine and make sure it is at neutral pH. Cysteine neutral solution needs to be freshly prepared and used the same day as prepared. Care must be taken so that the cysteine solution is at neutral pH and does not lower the pH of the digestion buffer. Prepare a stock solution of 1 M cysteine in double distilled water (90 µl aliquots may be stored at -20°C). To neutralize the cysteine solution thaw one vial and add 10 µl 8 M NaOH to the 90 µl cysteine solution. This gives 100 µl of 0.9 M pH neutral cysteine solution ready to use. **Note! Use freshly prepared (within 6 h), it cannot be stored.**

Preparation of IgG

- Prepare the mouse IgG in digestion buffer. The final IgG concentration should be in the range of 1-10 mg/ml.

Antibody Fragmentation

1. Reconstitute FabULOUS® in 40 µL double distilled H_2O to a concentration of 50 U/µL.
2. Add FabULOUS® to mIgG.
- Add 1 unit FabULOUS® / 1µg mIgG.
- Add cysteine to the reaction mixture to a final concentration of 30-50mM cysteine in the reaction.

3. Incubate for 1 hour[1] at 37°C.

Purification of fragments – CaptureSelect LC-kappa column

- Lids and bottom caps of microspin columns are used during the incubation.
- Before centrifugation of microspin columns remove the bottom caps and loosen the top lids.

4. Equilibration

- Break off the bottom seals of the CaptureSelect™ columns (save the cap) and loosen the lids.
- Remove the storage solution by centrifugation at 1000xg for 1min.
- Equilibrate the columns by adding 300 µl binding buffer and centrifuge the columns at 1000×g for 1min.
- Repeat the equilibration step twice.
- Seal the spin columns with the bottom caps.
- Immediately add the FabULOUS® digested sample to the CaptureSelect™ column and seal the columns with the top lids. Up to 0.5 mg digested IgG can be added to each column in a volume of 100-600 µl.
  Note! Minimum volume added to each column should be 100 µl to ensure proper mixing with the resin.

5. Binding of Fab fragments

- Take care to fully suspend the media, mix by inversion and make sure it is flowing in the column.
- Incubate the column by end-over-end mixing at room temperature for 30-60min.

6. Elution of Fc fragments

- Remove the bottom caps and loosen the lids.
- Place the columns in a 1.5-2 ml collection tubes.
- Centrifuge the columns at 1000×g for 1min to elute the Fc fragments.

Elution of Fab fragments – CaptureSelect LC-kappa column

7. Wash

- Add 300 µl binding buffer to the CaptureSelect™ columns.
- Centrifuge at 1000xg for 1min.
- Repeat twice.

8. Elution of Fab fragments

- Add 40 µl neutralizing buffer (0.1 volume) to each collection tube.
- Add 400 µl 0.1 M Glycine, pH 2.5 to each spin column and seal the columns.
- Take care to fully suspend the media by inversion of the spin columns a couple of times.

9. Collection of Fab fragments

- Immediately, loosen the lids and remove the bottom caps. Place the spin columns in the prepared collection tubes and centrifuge at 1000×g for 1 min to elute the Fab fragments.
- Repeat steps 8 and 9 twice for maximum recovery.
- Pool the eluted fractions.

Notes

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

Quality Control

FabULOUS® is tested to ensure lot-to-lot consistency.
FabULOUS® is tested for absence of microbial contamination with blood agar plates, Sabouraud dextrose agar plates and fluid thioglycollate medium

Customer Service USA Phone: 617-444-8421 I info@genovis.com I Genovis AB, 245 First Street suite 1800, Cambridge, MA 02142, USA
Customer Service Europe Phone: 0046 (0)46 10 12 30 I info@genovis.com I Genovis AB, Box 790, SE-220 07 Lund, Sweden
Support support@genovis.com **Order** order@genovis.com I Fax: 0046 (0)46 12 80 20

www.genovis.com