FabULOUS™ Fab kit

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STORE CONTENT AT DIFFERENT TEMPERATURES
(See page 8)

SmartEnzymes™

GENOVIS

INSTRUCTIONS FOR PRODUCT

FabULOUS™ Fab kit mouse (A1-PFK-020)
Digestion and purification of up to 2 mg mouse IgG

Quick Guide

- The Quick Guide (p. 3) is intended for experienced users. First time users are recommended to follow the detailed protocol (p. 8).
- Use lids and bottom caps during the incubation.
- Before centrifugation, remove the bottom cap and slightly open the lid.

Sample Preparation

- Prepare the antibody in 100-400 µl digestion buffer, 0.5 mg IgG for digestion and following purification per CaptureSelect™ LC-kappa (mur) column.

Last revised Feb 2021

Antibody Subunit Generation

1 Prepare FabULOUS™

- Reconstitute FabULOUS in 40 µl ddH_2O to a concentration of 50 units/µl.
- Prepare 0.9 M cysteine at neutral pH. *Note! Use the same day as it is prepared, it should not be stored.*

2 Add FabULOUS™

- Add 1 unit FabULOUS / 1 µg IgG.
- Add cysteine to a final concentration of 30-50 mM.

3 Digestion

- Incubate at 37°C for 1 hour.

Purification of Fragments – CaptureSelect™ LC-kappa (mur) Column

4 Equilibration

- Equilibrate the CaptureSelect™ column with 3 × 300 µl binding buffer. Centrifuge at 1000 × g for 1 min.

5 Binding of Fab Fragments

- Add the FabULOUS-digested sample from step 3.
- Cap the column and incubate by end-over-end mixing for 30-60 min at room temperature.

6 Collection of Fc Fragments

- Centrifuge at 1000 × g for 1 min to collect the Fc fragments. The FabULOUS enzyme is also present in this sample.

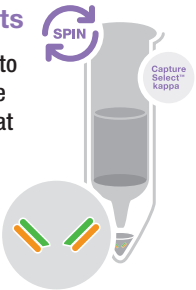
Elution of Fab Fragments – CaptureSelect™ LC-kappa (mur) Column

7 Wash

- Wash the CaptureSelect™ column with 3 × 300 µl binding buffer. Centrifuge at 1000 × g for 1 min.

8 Elution of Fab Fragments

- Add 40 µl neutralizing buffer (0.1 volume) to a collection tube.
- Add 400 µl 0.1 M glycine, pH 2.5, to the column, seal the column and invert manually a couple of times.



9 Collection of Fab Fragments

- Immediately transfer the column to the collection tube and collect the Fab fragments by centrifugation at 1000 × g for 1 min.
- For maximum recovery, repeat steps 8-9, 1-2 times and centrifuge at 1000 × g for 1 min.

PRODUCT DESCRIPTION

FabULOUS Fab kit mouse is designed for easy preparation of Fab fragments from mouse IgG antibodies. The preparation is performed in two steps, FabULOUS digestion of mouse IgG and purification of the fragments by using a LC-kappa (mur) affinity spin column.

The FabULOUS (SpeB) enzyme digests IgG from many species, including mouse, in the hinge region. FabULOUS is active on IgG under reducing conditions. By using optimized mild reducing conditions (30-50 mM cysteine) intact Fab fragments are generated. In presence of stronger reducing agents as DTT and TCEP the interchain thiols are likely reduced. FabULOUS digests IgG in commonly used buffers, with pH ranging from 6.5 to 8.0 (Table 1) and optimal activity is obtained at 37°C. Included in the kit are CaptureSelect™* LC-kappa (mur) affinity purification spin columns for easy purification of the generated Fab fragments from mouse IgG. 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 immunoglobulins which enables binding and purification of intact Fab fragments. If the IgG has LC-lambda, please contact the Genovis team. The CaptureSelect LC-lambda (mouse) is available upon request.

** 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.*

Content and Storage

- 1 × FabULOUS enzyme (2000 u), supplied lyophilized in Tris buffer saline (TBS) pH 7.6. One unit of FabULOUS digests ≥ 95% of 1 µg human IgG1 when incubated in PBS with 5 mM DTT or TCEP, pH 7.4 at 37°C for 1 hour. Valid also for 1 µg of mouse IgG1 in presence of 30-50 mM L-cysteine as reducing agent.
- 4 × CaptureSelect™ LC-kappa (mur) Microspin columns, one column includes sufficient material to purify up to 0.5 mg mouse IgG. The resin is supplied in 20% EtOH.

FabULOUS Fab kit mouse is shipped cold and the content should be stored at different temperatures:

- FabULOUS 2000 u should be stored at -20°C upon arrival.
- CaptureSelect™ LC-kappa resin in Microspin columns should be stored at +4-8°C. **Do not freeze the Microspin columns!**

After reconstitution, the FabULOUS enzyme is stable for at least 1 month at +4-8°C.

FabULOUS Fab kit mouse is for R&D use only.

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 ml and 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

Preparation of Cysteine

L-cysteine solution at neutral pH needs to be freshly prepared and used the same day. Prepare a stock solution of 1 M L-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.**

Sample Preparation

Prepare the mouse IgG in digestion 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.

Antibody Subunit Generation

1 Prepare FabULOUS™

- 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 / 1 µg mIgG.
- Add cysteine to the reaction mixture to a final concentration of 30-50 mM cysteine in the reaction.

3 Digestion

- Incubate the reaction mix for 1 hour[1] at 37°C.

Purification of Fragments – Each CaptureSelect™ LC-kappa (mur) Spin Column can Purify Fab from 0.5 mg mIgG

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

4 Equilibration

- Break off the bottom caps of the CaptureSelect™ columns (save the cap) and place the columns in collection tubes. Loosen the lids.
- Remove the storage solution by centrifugation at 1000 × g for 1 min.
- Equilibrate the columns by adding 300 µl binding buffer and centrifuge the columns at 1000 × g for 1 min.
- Repeat the equilibration step twice.
- Seal the spin columns with the bottom caps.

5 Binding of Fab Fragments

- Immediately add the FabULOUS digested sample from step 3 to the CaptureSelect™ columns 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 100-400 µl. **Note!** Minimum volume added to each column should be 100 µl to ensure proper mixing with the resin.
- Take care to fully suspend the media, mix by inversion and make sure there is a flow in the columns.

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

6 Collection of Fc Fragments

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

The FabULOUS enzyme is also present in this sample.

Elution of Fab Fragments – CaptureSelect™ LC-kappa (mur) Column

7 Wash

- Place the columns from step 6 in new collection tubes and add 300 µl binding buffer.
- Centrifuge at 1000 × g for 1 min. Discard the flow-through.
- Repeat wash twice.

8 Elution of Fab Fragments

- Seal the columns with the bottom caps.
- Prepare 2 ml collection tubes with 40 µl neutralizing buffer (0.1 × the elution volume).
- Add 400 µl 0.1 M glycine, pH 2.5 to each spin column and seal the columns with the lids.
- Fully suspend the media by manually inverting the columns a couple of times.

9 Collection of Fab Fragments

- Immediately remove the bottom caps of the columns and place the columns in the prepared 2 ml collection tubes. Loosen the lids. Centrifuge at 1000 × g to collect the Fab fragments.
- Repeat steps 8 and 9 twice for maximum recovery.
- Pool the collected Fab fractions.

Notes

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

Quality Control

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

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

Related Products

GingisKHAN® Fab kit
Generation and purification of Fab fragments from human IgG1

FabALACTICA®
Generation of Fab fragments from human IgG1

FabALACTICA® Fab kit
Generation and purification of intact Fab fragments from human IgG1

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