GingisKHAN® Fab kit

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STORE CONTENT AT DIFFERENT TEMPERATURES
(See page 7)

SmartEnzymes™

GENOVIS

INSTRUCTIONS FOR PRODUCT

GingisKHAN® Fab kit (B0-GFK-020)
Generation and purification of Fab fragments from up to 2 mg human IgG1

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 digestion buffer, 0.5 mg in 100-300 µl for digestion and following purification per CaptureSelect CH1 column.

Last revised Dec 2019

Antibody Subunit Generation

1 Prepare GingisKHAN®

- Reconstitute GingisKHAN in 200 µl ddH_2O to a concentration of 10 units/µl.
- Reconstitute GingisKHAN Reducing Agent in 50 µl ddH_2O[1] and keep on ice. *Note! Use the same day as it is prepared, it should not be stored.*

2 Add GingisKHAN®

- Add 1 unit GingisKHAN / 1 µg IgG.
- Add 1/10 v/v freshly prepared GingisKHAN Reducing Agent.

3 Digestion

- Incubate at 37°C for 1-2 hours[2].

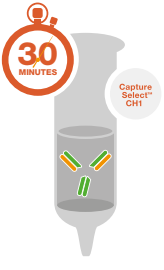
Purification of Fragments – CaptureSelect™ CH1 Column

4 Equilibration

- Equilibrate the CaptureSelect™ CH1 column with 3 x 300 µl binding buffer. Centrifuge at 200 x g for 1 min.

5 Binding of Fab Fragments

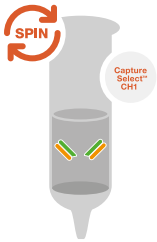
- Add the GingisKHAN-digested sample from step 3.
- Cap the column and incubate by end-over-end mixing for 30 min at room temperature.



6 Recovery of Fc Fragments[3]

- Centrifuge at 200 x g for 1 min to collect the Fc fragments.
- For maximum recovery, add 100 µl binding buffer and centrifuge at 200 x g for 1 min.
- Repeat once and centrifuge at 1000 x g for 1 min in the final centrifugation step.

Elution of Fab fragments – CaptureSelect™ CH1 Column

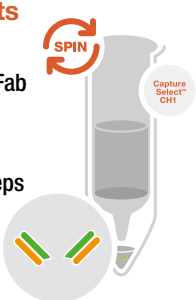


7 Wash

- Wash the CaptureSelect™ CH1 column with 2 x 300 µl binding buffer. Centrifuge at 200 x g for 1 min.

8 Elution of Fab Fragments

- Add 25 µl neutralizing buffer (0.1 volume) to a collection tube.
- Add 250 µl 0.1 M Glycine, pH 3.0, 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 200 x g for 1 min.
- For maximum recovery, repeat steps 8-9 once and centrifuge at 1000 x g for 1 min.

PRODUCT DESCRIPTION

GingisKHAN Fab kit is used for generation of pure Fab fragments from human IgG1. The kit involves two steps, GingisKHAN digestion of human IgG1, and purification of the fragments on an IgG-CH1-specific affinity spin column.

At native conditions, GingisKHAN digests human IgG1 at a single site in the upper hinge (…KSCDK / THTCPPCP…). A second digestion site on the Fc may appear if the N-glycans are removed. GingisKHAN is a cysteine protease that requires reducing conditions to be active. Intact Fab and Fc fragments are obtained with GingisKHAN digestion of human IgG1, since mild reducing conditions (2 mM cysteine) is sufficient for enzymatic activity. Optimal activity is obtained at 37°C and pH 8. The reducing agent (GingisKHAN Reducing Agent) is supplied together with the enzyme.

The CaptureSelect™* IgG-CH1 affinity resin recognizes the CH1 domain of human IgG, thereby enabling purification of the Fab fragments independent on the light-chain isotype. Due to its unique selectivity for the CH1 domain there will be no contamination of free light chain.

**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 x GingisKHAN (2000 units), is supplied lyophilized in 0.1 M Tris, 75 mM NaCl, pH 8.0. One unit of the GingisKHAN enzyme digests ≥ 95% of 1 µg human IgG1 when incubated in 0.1 M Tris, 2 mM cysteine, pH 8.0 at 37°C for 1 h.
- 5 x GingisKHAN Reducing Agent (10x), yielding 20 mM cysteine upon reconstitution (neutral pH).
- 4 x CaptureSelect™* IgG-CH1 Microspin columns, each column contains sufficient material to purify up to 0.5 mg IgG. Supplied in 20% EtOH.

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

GingisKHAN 2000 units and GingisKHAN Reducing Agent should be stored at -20°C upon arrival.

CaptureSelect™* IgG-CH1 Microspin columns should be stored at +4-8°C. **Do not freeze the microspin columns!**

After reconstitution, the GingisKHAN enzyme is stable for 2 months at +4-8°C. The reconstituted GingisKHAN Reducing Agent should be used the same day as it is prepared, it should not be stored.

GingisKHAN Fab kit is for R&D use only.

- Use lids and bottom caps of microspin columns during the incubation.
- Before centrifugation of the microspin columns, remove the bottom cap (save the cap) and slightly loosen the lid (do not remove the lid).

Additional Materials Required

- Digestion buffer[4]: 0.1 M Tris, pH 8.0
- Binding buffer: PBS or TBS, pH 7.0-7.5 (physiological pH and ionic strength)
- Elution buffer: 0.1 M Glycine, pH 3.0
- Neutralizing buffer: 1 M Tris, pH 8.0
- Reaction/Collection tubes: Microcentrifuge tubes (1.5-2 ml).

Sample Preparation

- Prepare the human IgG1 in digestion buffer. If 2 mg of an antibody is processed at a time, the volume can be 400-1200 µl. Digest from 0.5 mg antibody can be purified on one CaptureSelect™ CH1 column.

Antibody Subunit Generation

1 Prepare GingisKHAN®

- Reconstitute GingisKHAN in 200 µl ddH_2O to a concentration of 10 units/µl.
- Reconstitute GingisKHAN Reducing Agent in 50 µl ddH_2O[1] and keep on ice. **Note! Use the same day as it is prepared, it should not be stored.**

2 Add GingisKHAN® to the IgG

- Add 1 unit GingisKHAN / 1 µg IgG.
- Add GingisKHAN Reducing Agent to the reaction mixture. Add 1/10 (v/v) to yield 2 mM cysteine in the reaction.

3 Digestion

- Incubate the reaction mix at 37°C for 1-2 hours[2].

Purification of Fragments – Each CaptureSelect™ CH1 Spin Column can Purify Fab from 0.5 mg IgG.

4 Equilibration

- Break off the bottom cap of the column (save the cap) and place the column in a collection tube. Loosen the lid.
- Centrifuge at 200 x g for 1 min to remove the storage solution.
- Equilibrate the column by adding 300 µl binding buffer and centrifuge the column at 200 × g for 1 min.
- Repeat the equilibration step twice.
- Seal the spin column with the bottom cap.

5 Binding of the Fab Fragments

- Immediately add the GingisKHAN-digested sample from step 3 to the equilibrated column and seal the column with the top lid.
  Up to 0.5 mg digested IgG can be added to each column in a volume of 100-400 µl.

Note! The minimum volume added to each column should be 100 µl to ensure proper mixing with the resin.

- Fully suspend the media, mix it manually by inversion a couple of times.
- Incubate the column by end-over-end mixing at room temperature for 30 min and make sure there is a flow in the column.

6 Collection of the Fc Fragments

- Remove the bottom cap and place the column in a collection tube. Loosen the lid.
- Centrifuge the column at 200 × g for 1 min to collect the Fc fragments.

For Maximum Recovery of Fc Fragments:

- Seal the column with the bottom cap.
- Add 100 µl binding buffer, seal the column with the top lid and invert a couple of times.
- Remove the bottom cap and place the column in a collection tube. Loosen the lid.
- Centrifuge at 200 × g for 1 min to collect the Fc fragments.
- Repeat once. Centrifuge at 1000 × g for 1 min in the final centrifugation step.
- Pool the Fc fractions[3].

Elution of Fab Fragments – CaptureSelect™ CH1 Spin Column

7 Wash

- Add 300 µl binding buffer to the column from step 6, remove the bottom cap and place the column in a collection tube.
- Centrifuge at 200 x g for 1 min. Discard the flow-through.
- Repeat once.

8 Elution of Fab Fragments

- Seal the column with the bottom cap.
- Prepare a collection tube with 25 µl neutralizing buffer (0.1 x the elution volume).
- Add 250 µl 0.1 M Glycine, pH 3.0 to the column and seal the column with the lid.
- Fully suspend the media by manually inverting the column a couple of times.

9 Collection of Fab Fragments

- Immediately remove the bottom cap of the column and place the column in the prepared collection tube. Loosen the lid. Centrifuge at 200 x g for 1 min to collect the Fab fragments.
- Repeat steps 8 and 9 for maximum recovery. Centrifuge at 1000 × g for 1 min in the final centrifugation step.
- Pool the collected Fab fractions.

Notes

1. *Upon reconstitution, the GingisKHAN Reducing Agent can appear cloudy. This will not affect its performance. Make sure to mix it thoroughly before adding it to the reaction.*
2. *The digestion time may need to be optimized for individual antibodies.*
3. *If intact Fc fragments are to be used, a desalting step is needed since the eluate contains Reducing Agent from the Fragmentation step. GingisKHAN enzyme will be present in the Fc fraction.*
4. *Other buffers at pH 7-8 can be used, but optimization is required. Sodium chloride concentrations above 75 mM may negatively affect enzymatic activity.*

Generation of Fab fragments from hIgG1 with GingisKHAN is adversely affected at denaturing conditions i.e. in the presence of chaotropic agents and/or detergents. If the analysis of the digestion efficiency is done with SDS-PAGE, stop the digestion reaction by adding 10 mM iodoacetamide before SDS is added to the SDS-PAGE sample preparation.

Quality Control

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

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

Related Products

GingisKHAN®
Digestion of up to 2 mg 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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