

INSTRUCTIONS

Version 17.1.1

Instructions for Product

GingisKHAN® Fab kit

(B0-GFK-020)

Digestion and purification of up to 2mg human IgG1

Product Description

GingisKHAN Fab kit is used to generate purified Fab fragments. The kit involves two steps, GingisKHAN digestion of IgG and purification of the fragments on a IgG-CH1 specific affinity spin column.

GingisKHAN (Gingipain K) is an enzyme that digests human IgG1 yielding intact Fab and Fc fragments. GingisKHAN digests human IgG1 at one single site in the upper hinge (...KSCDK / THTCPPCP...). A second cleavage site on the Fc may appear if the N-glycans are removed. GingisKHAN is a cysteine protease and requires reducing environment to be active. Intact Fab and Fc fragments are obtained with GingisKHAN digestion of human IgG1 since mild reducing conditions (i.e. 2 mM cysteine) is sufficient for enzyme activity. Optimal activity is obtained at 37°C and pH 8. Reducing agent is supplied with the enzyme.

The CaptureSelect™ IgG-CH1* affinity matrix recognizes the CH1 domain of human IgG antibodies, which enables purification of Fab fragments, independent of the light-chain isotype and source material. Due to its unique selectivity for the CH1 domain, no co-purification of free light-chain contaminants will occur.

Content and storage

- 1x GingisKHAN 2000u, supplied as a lyophilized powder formulated in Tris/NaCl. One unit of GingisKHAN enzyme digests ≥ 95% of 1µg human IgG1 when incubated in 0.1 M Tris, pH 8.0 at 37°C for 1h.
- 5x GingisKHAN Reducing Agent (10x), containing 20 mM cysteine upon reconstitution.
- 4x CaptureSelect IgG-CH1* MicroSpin columns, each column includes sufficient material to purify up to 0.5 mg IgG. It is supplied in 20% EtOH.

GingisKHAN Fab kit is shipped on ice.

GingisKHAN 2000u and GingisKHAN Reducing Agent should be stored at -20°C upon arrival. CaptureSelect™ IgG-CH1 resin* in MicroSpin columns should be stored in +4-8°C.

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

GingisKHAN 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.

Protocol

Additional Material Supplied

GingisKHAN Reducing Agent (10x).

Additional Materials Required

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

Sample Pre-treatment

- Prepare the IgG (human IgG1) in digestion buffer. The final IgG concentration should be in the range of 1-10 mg/ml.

Antibody Fragmentation by GingisKHAN™

1. **Prepare GingisKHAN.**
 - Reconstitute GingisKHAN in 200µL double distilled H₂O to a concentration of 10 U/µL.
 - Reconstitute GingisKHAN Reducing Agent in 50µL double distilled H₂O and keep on ice. Note! Use the same day as prepared, it cannot be stored.
2. **Add GingisKHAN™ to IgG.**
 - Add 1 unit GingisKHAN / 1µg IgG.
 - Add GingisKHAN Reducing Agent to the reaction mixture. Add 1/10 v/v to yield 2mM cysteine in reaction.
3. **Digestion.**
 - Incubate for 1-2 hours² in digestion buffer at 37°C.

Purification of fragments – CaptureSelect CH1 column

- Lids and bottom caps of microspin columns are used during the incubation.
- Before centrifugation of microspin columns remove the bottom cap and slightly open the lid ~90° counter clockwise.

4. Equilibration

- Break off the bottom seal of the CaptureSelect™ column (save the cap) and slightly open the lids ~90° counter clockwise.
- Remove the storage solution by centrifugation at 200xg for 1min.
- Equilibrate the column by adding 300µl binding buffer and centrifuge the column at 200xg for 1min.
- Repeat the equilibration step twice.
- Seal the spin column with the bottom cap.
- Immediately add the GingisKHAN digested sample (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 volume in a volume of 100-400µl.
Note! Minimum volume added to each column should be 100µl to ensure proper mixing with the resin.

5. Binding of Fab fragments

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

6. Elution of Fc fragments³

- Remove the lids and the bottom caps.
- Place the column in a 1.5-2 ml collection tube.
- Centrifuge the columns at 200xg for 1min to elute the Fc fragments.

For maximum recovery of Fc fragments:

- Add 100µl binding buffer to the column and place the column in a new 1.5-2 ml collection tube.
- Centrifuge the column at 200xg for 1min to elute the Fc fragments.
- Repeat once. Centrifuge at 1000xg for 1min in the final centrifugation step.
- Pool the eluted fractions.

Elution of Fab fragments – CaptureSelect CH1 column

7. Wash

- Add 300µl binding buffer to the CaptureSelect™ column.
- Centrifuge at 200xg for 1min.
- Repeat once.

8. Elution of Fab fragments

- Add 25µl neutralizing buffer (0.1 volume) to each collection tube.
- Add 250µl 0.1M Glycine, pH 3.0 to each spin column and seal the columns.
- Take care to fully suspend the media by manual inversion of the spin columns a couple of times.

9. Collection of Fab fragments

- Immediately, open the lids and remove the bottom caps. Place the spin columns in the prepared collection tubes and centrifuge at 200xg for 1min to elute the Fab fragments.
- Repeat steps 8 and 9 once for maximum recovery. Centrifuge at 1000xg for 1min in the final centrifugation step.
- Pool the eluted fractions.

Notes

1. Upon reconstitution, the Reducing Agent can appear cloudy. This will not affect its performance. Take care to mix it thoroughly before adding to the reaction.
2. 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 this elute contains Reducing Agent from the Fragmentation step.

Digestion of human IgG1 by GingisKHAN is negatively affected at denaturing conditions i.e. in the presence of chaotropic agents and/or detergents. If analysis of digestion efficiency is done with SDS-PAGE take care to run samples immediately after SDS sample preparation. Preferentially, stop the reaction with 10 mM iodoacetamide before SDS sample preparation.

GingisKHAN®

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