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GingisKHAN™

Fab Kit

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Instructions for Use

GingisKHAN™ Fab Kit 2 mg (B0-GFK-020)
Process 2 mg hIgG1

DOWNLOAD INSTRUCTIONS FOR USE
www.genovis.com/ifu-B0-GFK

Lyophilized Enzyme and Affinity Resin for Above Hinge Digestion of Human IgG1 and Purification of Fab Fragments

GingisKHAN (Kgp) is a cysteine protease that digests human IgG1 at a single site above the hinge, generating intact and homogenous Fab and Fc fragments.[1]

GingisKHAN Fab Kit consists of GingisKHAN Lyophilized and GingisKHAN Reducing Agent for digestion of human IgG1, and spin columns with CaptureSelect™* CH1 resin for affinity purification of Fab fragments. GingisKHAN requires mildly reducing conditions (2 mM L-cysteine) to be active, and optimal activity is obtained at 37°C and pH 8.0. The reducing agent is provided together with the enzyme.

GingisKHAN is purified from *Porphyromonas gingivalis*.

The CaptureSelect™ IgG-CH1 affinity matrix recognizes the CH1 domain of human IgG, enabling purification of Fab fragments independent on the light-chain isotype. Due to its unique selectivity for the CH1 domain, no co-purification of free light-chain contaminants will occur.

CONTENT AND STORAGE

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

- **1 vial GingisKHAN Lyophilized** is supplied lyophilized in 100 mM Tris, 75 mM NaCl, pH 8.0, with no preservatives added. One unit GingisKHAN Lyophilized digests ≥ 95% of 1 µg human IgG1 when incubated in 0.1 M Tris, pH 8.0 at 37°C in presence of 2 mM L-cysteine for 1 hour. GingisKHAN Lyophilized should be stored at -20°C upon arrival. After reconstitution, GingisKHAN Lyophilized is stable for 2 months at +4-8°C.
- **5 vials GingisKHAN Reducing Agent** (10×) is supplied lyophilized, yielding 20 mM L-cysteine upon reconstitution (neutral pH).
- **4 CaptureSelect™ IgG-CH1 Microspin columns** are supplied in 20% ethanol with no preservatives added. One column contains sufficient material to purify 0.5 mg hIgG1. The columns should be stored at +4-8°C upon arrival. **Do not freeze the columns!**

GingisKHAN Fab Kit is for R&D use only.

QUALITY CONTROL

GingisKHAN Lyophilized included in GingisKHAN Fab Kit is tested to meet the specifications and lot-to-lot consistency.

GingisKHAN 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

FabRICATOR® Xtra
Below hinge digestion of mutated IgG

FabALACTICA™
Above hinge digestion of human IgG1

* 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.

Preparations

Important Information

- Use lids and bottom caps during the incubation.
- Before centrifugation, remove the bottom cap and loosen the lid (do not remove the lid).
- Please note that reconstituted GingisKHAN Reducing Agent cannot be stored – **use it freshly prepared!**

Additional Materials Required

- Reaction buffer: 0.1 M Tris, pH 8.0.[2]
- 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.
- Microcentrifuge tubes: 1.5-2 ml.

Above Hinge Digestion of Human IgG1

Sample Preparation
Prepare the human IgG1 in the reaction buffer. If 2 mg antibody is processed at a time, the volume can be 400-1000 µl. Digest from 0.5 mg antibody in 100-250 µl can be purified on one CaptureSelect™ CH1 column.

1. Prepare GingisKHAN Lyophilized

1.1 Reconstitute GingisKHAN Lyophilized in 200 µl ddH_2O to a concentration of 10 units/µl.

1.2 Reconstitute GingisKHAN Reducing Agent in 50 µl ddH_2O and keep on ice.[3] **Note! Use the same day as it is prepared, it cannot be stored.**

2. Add GingisKHAN Lyophilized

2.1 Add 1 unit GingisKHAN Lyophilized / 1 µg IgG.

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

3. Enzymatic Reaction

3.1 Incubate for 1-2 hours[4] at 37°C.

1. GingisKHAN is a lysine-specific protease, and the digestion site on human IgG1 is …KSCDK / THTCPPC… The single digestion site is a result of the three-dimensional structure of human IgG1, making this lysine exposed for the enzyme. Additional digestion sites at exposed lysines on the Fc may appear if the N-glycans are removed.
2. Other buffers at pH 7-8 can be used, but optimization is required. Sodium chloride concentrations above 75 mM may negatively affect enzymatic activity.
3. Upon reconstitution, the GingisKHAN Reducing Agent may appear cloudy. This does not affect the performance. Make sure to mix it thoroughly before using it in the reaction.
4. Digestion time may need to be optimized for individual antibodies.

Purification of Fab Fragments

4. Equilibration

4.1 Break off the bottom cap of the CaptureSelect™ IgG-CH1 column[5] (save the cap) and place the column in a microcentrifuge tube. Loosen the lid.

4.2 Centrifuge at 200 × 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 200 × 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 sample from step 3.1 to the CaptureSelect™ IgG-CH1 column and seal the column with the lid. 0.5 mg IgG can be added to each column in a volume of 100-250 µl. **Note!** The 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 min.

6. Recovery of Fc Fragments

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

6.2 Centrifuge at 200 × g for 1 min to collect the Fc fragments.

7. For Maximum Recovery of the Sample

7.1 Insert the bottom cap.

7.2 Add 100 µl binding buffer, seal the column with the lid and invert it a couple of times.

7.3 Remove the bottom cap and place the column in a new microcentrifuge tube. Loosen the lid.

7.4 Centrifuge at 200 × g for 1 min to collect the Fc fragments.

7.5 Repeat steps 7.1-7.4. In step 7.4, centrifuge at 1000 × g for 1 min.

7.6 Pool the collected Fc fragments, including the sample from step 6.2.[6]

5. Four CaptureSelect IgG-CH1 Microspin columns are included. Each column contains sufficient material to purify 0.5 mg human IgG1.

6. If intact Fc fragments are to be used, a desalting step is needed since the solution contains Reducing Agent from the digestion step. GingisKHAN enzyme will be present in the Fc fraction.

Elution of Fab Fragments

8. Wash

8.1 Place the column from step 7.6 in a new microcentrifuge tube and add 300 µl binding buffer.

8.2 Centrifuge at 200 × g for 1 min. Discard the flow-through.

8.3 Repeat steps 8.1-8.2.

9. Elution of Fab Fragments

9.1 Insert the bottom cap.

9.2 Prepare a new microcentrifuge tube with 25 µl neutralizing buffer.

9.3 Add 250 µl 0.1 M Glycine, pH 3.0 to the column and seal the column with the lid.

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

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

9.6 Centrifuge at 200 × g for 1 min to collect the eluted Fab fragments.

9.7 Repeat steps 9.1-9.6 for maximum recovery. In step 9.6, centrifuge at 1000 × g for 1 min.

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

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