

# GingisKHAN®

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FOR RESEARCH  
USE ONLY  
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STORE AT  
-20°C



SmartEnzymes™

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## INSTRUCTIONS FOR PRODUCT

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**GingisKHAN® 2000 units** (B0-GKH-020)

Digestion of up to 2 mg human IgG1

## 1 Prepare GingisKHAN®

- Reconstitute GingisKHAN in 200  $\mu$ l ddH<sub>2</sub>O to a concentration of 10 units/ $\mu$ l.
- Reconstitue GingisKHAN Reducing Agent in 50  $\mu$ L ddH<sub>2</sub>O<sup>1</sup>.



## 2 Add GingisKHAN®

- Add 1 unit GingisKHAN / 1  $\mu$ g IgG.
- Add 1/10 (v/v) freshly prepared GingisKHAN Reducing Agent.



## 3 Digestion

Incubate for 1-2 h<sup>2</sup> at 37°C.



# PRODUCT DESCRIPTION

GingisKHAN (Gingipain K) is an enzyme that digests human IgG1, yielding intact Fab and Fc fragments. In native conditions, GingisKHAN digests human IgG1 at one single site in the upper hinge (Table 1).

A second digestion site on the Fc may appear if the N-glycans are removed. GingisKHAN is a cysteine protease and requires reducing conditions 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. The enzyme can be deactivated by addition of iodoacetamide. The reducing agent is supplied together with the enzyme.

GingisKHAN is purified from *Porphyromonas gingivalis*.

**Table 1.** *GingisKHAN digestion site.*

<b>IgG subclass</b>	<b>GingisKHAN digestion site</b>
Human IgG1	..KSCDK / THTCPPCP..

## Unit Definition

One unit GingisKHAN digests  $\geq 95\%$  of 1  $\mu\text{g}$  human IgG1 when incubated in 0.1 M Tris, pH 8.0 at 37°C for 1 h.

## Content and Storage

GingisKHAN is supplied lyophilized in 100 mM Tris, 75 mM NaCl, pH 8.0, with no preservatives added. It is supplied with 5 vials of lyophilized GingisKHAN Reducing Agent (10x), yielding 20 mM cysteine upon reconstitution (neutral pH).

GingisKHAN and GingisKHAN Reducing Agent are shipped cold and should be stored at -20°C upon arrival.

After reconstitution, GingisKHAN is stable for 2 months at +4-8°C.

Reconstituted GingisKHAN Reducing Agent should be used the same day as it is prepared, it cannot be stored.

GingisKHAN is for R&D use only.

# DETAILED PROTOCOL

## **Additional Materials Supplied**

- GingisKHAN Reducing Agent (10x), 5 vials.

## **Additional Materials Required**

- Digestion buffer<sup>3</sup>: 0.1 M Tris, pH 8.0.

## **Preparations**

- Prepare the IgG (human IgG1) in digestion buffer. The final IgG concentration should be 0.5-5 mg/ml.
- GingisKHAN Reducing Agent must be prepared and used the same day, it cannot be stored<sup>1</sup>.

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 analysis of the digestion efficiency is done with SDS-PAGE, stop the digestion reaction in the SDS-PAGE sample preparation by adding iodoacetamide (to 10 mM) before SDS is added.

## Antibody Subunit Generation

### 1 Prepare GingisKHAN<sup>®</sup>

- Reconstitute GingisKHAN in 200  $\mu$ l ddH<sub>2</sub>O to a concentration of 10 units/ $\mu$ l.
- Reconstitute GingisKHAN Reducing Agent in 50  $\mu$ l ddH<sub>2</sub>O and keep on ice<sup>1</sup>.  
**Note! Use the same day as it is prepared, it cannot be stored.**

### 2 Add GingisKHAN<sup>®</sup>

- Add 1 unit GingisKHAN/ 1  $\mu$ g 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 for 1-2 hours<sup>2</sup> at 37°C.

#### Notes

1. 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.
2. Digestion time may need to be optimized for individual antibodies.
3. Other buffers at pH 7-8 can be used, but optimization is required. Sodium chloride concentrations above 75 mM may negatively affect enzymatic activity.

## 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® Fab kit**

Generation and purification of Fab fragments from hlgG1

### **FabALACTICA®**

Generation of Fab fragments from hlgG1



**GingisKHAN®**

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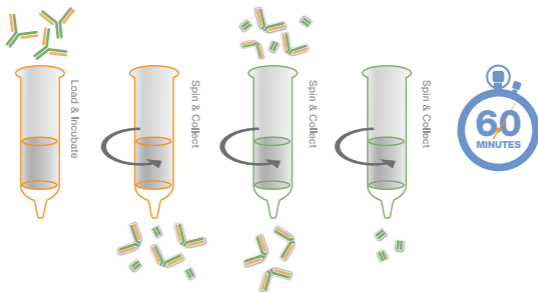
Genovis products are intended for research use only. They are not intended to be used for therapeutic or diagnostic purposes in humans or animals.

## FragIT™ kit

### Digestion of IgG and Purification of F(ab')<sub>2</sub> and Fc Fragments

FragIT kit consists of an IgG digestion column, FragIT, and an affinity purification column, CaptureSelect™\*. FragIT is a resin with FabRICATOR® enzyme covalently coupled to agarose beads for digestion of IgG to generate F(ab')<sub>2</sub> and Fc fragments. After digestion, the fragments can easily be purified using the CaptureSelect™\* column supplied in the kit.

- Digestion of IgG on a column.
- Purification of F(ab')<sub>2</sub> and Fc fragments.



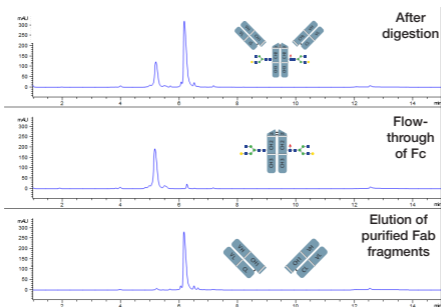
*\*Made with Thermo Scientific™ CaptureSelect™ resin from Thermo Fisher Scientific Inc. and its subsidiaries. Thermo Scientific and CaptureSelect are trademarks of Thermo Fisher Scientific Inc. and its subsidiaries.*

# GingisKHAN<sup>®</sup> Fab kit

## Generation and Purification of Fab Fragments from Human IgG1

GingisKHAN Fab Kit consists of GingisKHAN enzyme for upper hinge digestion of human IgG1 and an affinity column for purification of Fab fragments. After digestion, the Fab fragments can easily be purified using the CaptureSelect<sup>™</sup> CH1\* specific spin column supplied in the kit.

- Digestion of human IgG1 above the hinge.
- Purification of intact Fab fragments.



*\*Made with Thermo Scientific<sup>™</sup> CaptureSelect<sup>™</sup> resin from Thermo Fisher Scientific Inc. and its subsidiaries. Thermo Scientific and CaptureSelect are trademarks of Thermo Fisher Scientific Inc. and its subsidiaries.*



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