

## INSTRUCTIONS

Version 15.1.5

### Instructions for Product

GingisKHAN™ 2000 units (B0-GKH-020) Digestion of up to 2mg human IgG1

### Product Description

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.

### Unit Definition

One unit digests  $\geq 95\%$  of 1 $\mu$ g human IgG1 when incubated in 0.1 M Tris, pH 8.0 at 37°C for 60 min.

### Additional Material Supplied

- GingisKHAN™ Reducing Agent (10x).

### Additional Material Required

- Digestion buffer: 0.1 M Tris, pH 8.0.

### Content and Storage

GingisKHAN™ is formulated in 0.1 M Tris, 75 mM NaCl, pH 8.0 and supplied as a lyophilized powder. It is supplied with 5 vials of lyophilized GingisKHAN™ reducing agent (10x) containing 20 mM cysteine upon reconstitution.

GingisKHAN™ and GingisKHAN™ Reducing Agent are shipped on ice. Vials should be stored at -20°C upon arrival.

After reconstitution GingisKHAN™ is stable for at least 1 month +4-8°C. Reconstituted GingisKHAN™ Reducing Agent should be used immediately and cannot be stored.

GingisKHAN™ is for R&D use only.

### Quality Control

GingisKHAN™ is tested to ensure lot-to-lot consistency.

**Protocol** Prepare the IgG (human IgG1) in digestion buffer. The final IgG concentration should be in the range of 0.5-5 mg/ml.

### Antibody Fragmentation by GingisKHAN™

- Reconstitute GingisKHAN™ in 200  $\mu$ L double distilled H<sub>2</sub>O to a concentration of 10 U/  $\mu$ L.
- Reconstitute GingisKHAN™ Reducing Agent in **50  $\mu$ L** double distilled H<sub>2</sub>O. **Note! Use freshly prepared, it cannot be stored.**
- Add **1 unit** GingisKHAN™/ **1  $\mu$ g IgG**.
- Add GingisKHAN™ Reducing Agent to the reaction mixture. Add 1/10 v/v to yield 2mM cysteine in reaction.
- Incubate for **1-2 hours**<sup>1</sup> in digestion buffer at **37°C**.

### Notes

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

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, run the SDS-Page under reducing conditions or stop the reaction with 10 mM iodoacetamide before SDS sample preparation.

### GingisKHAN™

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