



# GingisKHAN™

Lyophilized

STORE AT

**-20°C**



FOR RESEARCH USE ONLY

## Instructions for Use

GingisKHAN™ Lyophilized 2000 units (B0-GKH-020)  
Process 2 mg human IgG1

DOWNLOAD INSTRUCTIONS FOR USE



[www.genovis.com/ifu-B0-GKH](http://www.genovis.com/ifu-B0-GKH)

## **Lyophilized Enzyme for Above Hinge Digestion of Human IgG1**

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.

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. 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*.

### **UNIT DEFINITION**

One unit GingisKHAN Lyophilized digests  $\geq 95\%$  of 1  $\mu\text{g}$  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.

### **CONTENT AND STORAGE**

GingisKHAN Lyophilized contains two components. The product box is shipped cold, and the two components should be stored at -20°C upon arrival.

- **1 vial GingisKHAN Lyophilized** is supplied lyophilized in 100mM Tris, 75mM NaCl, pH 8.0, with no preservatives added. After reconstitution, GingisKHAN Lyophilized is stable for 2 months at +4-8°C.
- **5 vials GingisKHAN Reducing Agent (10x)** is supplied lyophilized, yielding 20mM L-cysteine upon reconstitution (neutral pH). Please note that reconstituted GingisKHAN Reducing Agent cannot be stored – **use it freshly prepared!**

GingisKHAN Lyophilized is for R&D use only.

### **QUALITY CONTROL**

GingisKHAN Lyophilized 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**

#### **GingisKHAN™ Fab Kit**

Lyophilized enzyme and affinity resin for above hinge digestion of human IgG1 and purification of Fab fragments.

#### **FabALACTICA™**

Above hinge digestion of human IgG1

#### **FabDELLO®**

Above hinge digestion of human IgG1, including hinge-mutated IgG

## Preparations

### Important Information

- The GingisKHAN Reducing Agent must be prepared and used the same day, it cannot be stored.<sup>1</sup>

### Additional Materials Required

- Reaction buffer: 0.1 M Tris, pH 8.0.<sup>2</sup>

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. Other buffers at pH 7.0-8.0 can be used, but optimization is required. NaCl concentrations above 75 mM reduce the enzymatic activity.

## Above Hinge Digestion of Human IgG1

### Sample Preparation

- Prepare the human IgG1 in the reaction buffer. The final IgG concentration in the reaction should be 0.5-5 mg/ml.
- 1. Prepare GingisKHAN**
    - 1.1 Reconstitute GingisKHAN Lyophilized in 200  $\mu$ l ddH<sub>2</sub>O to a concentration of 10 units/ $\mu$ l.
    - 1.2 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**
    - 2.1 Add 1 unit GingisKHAN Lyophilized / 1  $\mu$ g IgG.
    - 2.2 Add GingisKHAN Reducing Agent to the reaction mixture. Add 1/10 (v/v) to yield 2 mM L-cysteine in the reaction.
  - 3. Enzymatic Reaction**
    - 3.1 Incubate for 1-2 h<sup>3</sup> at 37°C.

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



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