

## Product Information

**HGK (1-328), active, GST tagged, human  
PRECISIO® Kinase  
recombinant, expressed in *Sf9* cells**

Catalog Number **SRP5038**  
Storage Temperature  $-70^{\circ}\text{C}$

Synonyms: MAP4K4, NIK, FLH21957, FLJ10410,  
FLJ20373, FLJ90111, KIAA0687

### Product Description

HGK is the mitogen-activated protein kinase kinase kinase 4 (MAP4K4) and a member of the serine/threonine protein kinase family. HGK has been shown to specifically activate MAPK8/JNK.<sup>1</sup> The activation of MAPK8 by HGK can be inhibited by dominant-negative mutants of MAP3K7/TAK1, MAP2K4/MKK4, and MAP2K7/MKK7, which suggests that this kinase functions through the MAP3K7-MAP2K4-MAP2K7 kinase cascade and mediates TNF- $\alpha$  signaling. HGK-dependent signaling inhibits PPAR $\gamma$ -responsive gene expression, adipogenesis, and insulin-stimulated glucose transport.<sup>2</sup>

Recombinant human HGK (1-328) was expressed by baculovirus in *Sf9* insect cells using an N-terminal GST tag. The protein accession number is NP\_004825. Recombinant protein stored in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM glutathione, 0.1 mM EDTA, 0.25 mM DTT, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~64 kDa

Purity: 70–95% (SDS-PAGE, see Figure 1)

Specific Activity: 4.1–5.5 nmole/min/mg (see Figure 2)

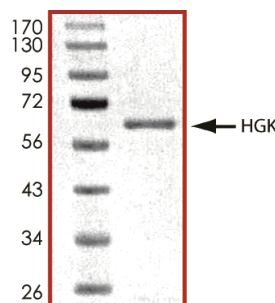
### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

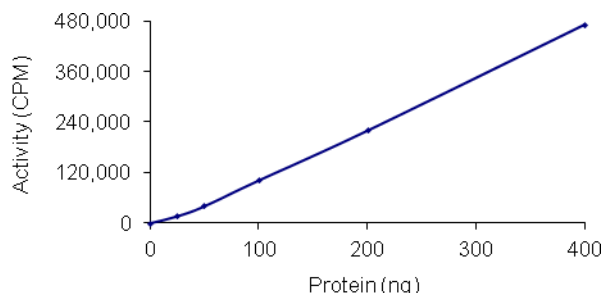
### Storage/Stability

The product ships on dry ice and storage at  $-70^{\circ}\text{C}$  is recommended. After opening, aliquot into smaller quantities and store at  $-70^{\circ}\text{C}$ . Avoid repeated handling and multiple freeze/thaw cycles.

**Figure 1.**  
SDS-PAGE Gel of Typical Lot  
70–95% (densitometry)



**Figure 2.**  
Specific Activity of Typical Lot  
4.1–5.5 nmole/min/mg



### Procedure

#### Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM MgCl<sub>2</sub>, 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with a 50 ng/μl BSA.

Kinase Solution – Dilute the active HGK (0.1 µg/µl) with Kinase Dilution Buffer to the desired concentration.

**Note:** The specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active HGK kinase for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200 µl aliquots at –20 °C.

γ-<sup>33</sup>P-ATP Assay Cocktail (250 µM) – Combine 5.75 ml of Kinase Assay Buffer, 150 µl of 10 mM ATP Stock Solution, 100 µl of γ-<sup>33</sup>P-ATP (1 mCi/100 µl). Store in 1 ml aliquots at –20 °C.

Substrate Solution – Dissolve the Myelin Basic Protein substrate in distilled water at a final concentration of 1 mg/ml.

1% phosphoric acid solution – Dilute 10 ml of concentrated phosphoric acid to a final volume of 1 L with water.

#### Kinase Assay

This assay involves the use of the <sup>33</sup>P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active HGK, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ-<sup>33</sup>P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 20 µl:
  - 10 µl of Kinase Solution
  - 5 µl of Substrate Solution
  - 5 µl of cold water (4 °C)
3. Set up a blank control as outlined in step 2, substituting 5 µl of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 µl of the γ-<sup>33</sup>P-ATP Assay Cocktail, bringing the final reaction volume to 25 µl. Incubate the mixture in a water bath at 30 °C for 15 minutes.
5. After the 15 minute incubation, stop the reaction by spotting 20 µl of the reaction mixture onto an individually pre-cut strip of phosphocellulose P81 paper.

6. Air dry the pre-cut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of ~10 minutes each.
7. Set up a radioactive control to measure the total γ-<sup>33</sup>P-ATP counts introduced into the reaction. Spot 5 µl of the γ-<sup>33</sup>P-ATP Assay Cocktail on a pre-cut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 3) from each sample and calculate the kinase specific activity

#### Calculations:

1. Specific Radioactivity (SR) of ATP (cpm/nmole)

$$SR = \frac{\text{cpm of 5 } \mu\text{l of } \gamma\text{-}^{33}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

cpm – value from control (step 7)

nmole – 1.25 nmole (5 µl of 250 µM ATP Assay Cocktail)

2. Specific Kinase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta\text{cpm} \times (25/20)}{SR \times E \times T}$$

SR = specific radioactivity of the ATP (cpm/nmole ATP)

Δcpm = cpm of the sample – cpm of the blank (step 3)

25 = total reaction volume

20 = spot volume

T = reaction time (minutes)

E = amount of enzyme (mg)

#### References

1. Yao, Z. et.al., A novel human STE20-related protein kinase, HGK, that specifically activates the c-jun N-terminal kinase signaling pathway. J. Biol. Chem., **274**, 2118-2125 (1999).
2. Tang, X. et.al., An RNA interference-based screen identifies MAP4K4/NIK as a negative regulator of PPAR-gamma, adipogenesis, and insulin-responsive hexose transport. Proc. Nat. Acad. Sci., **103**, 2087-2092 (2006).

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