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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
M26-11G-05	5 µg
M26-11G-10	10 µg

HGK, Active

Human recombinant protein expressed in Sf9 cells

Catalog # M26-11G

Lot # K1563-4

Product Description

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.

Gene Aliases

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

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, and 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

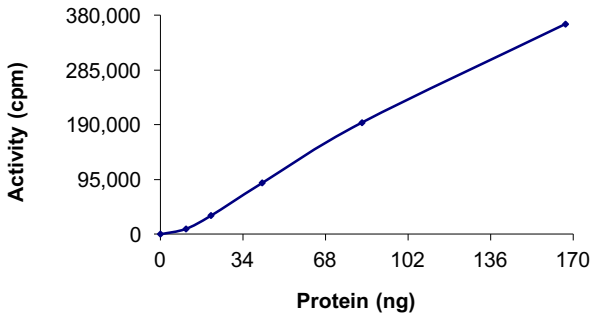
Scientific Background

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

References

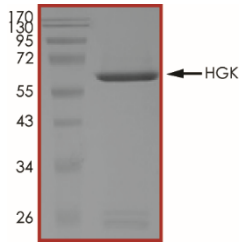
1. Yao, Z. et.al: A novel human STE20-related protein kinase, HGK,that specifically activates the c-jun N-terminal kinase signaling pathway. J. Biol. Chem. 274: 2118-2125, 1999.
2. Tang, X. et.al: An RNA interference-based screen identifies MAP4K4/NIK as a negative regulator of PPAR-gamma, adipogenesis,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

Specific Activity



The specific activity of HGK was determined to be **61 nmol /min/mg** as per activity assay protocol.

Purity



The purity of HGK was determined to be **>95%** by densitometry, approx. MW **~64kDa**.

HGK, Active

Human recombinant protein expressed in Sf9 cells

Catalog #	M26-11G
Specific Activity	61 nmol/min/mg
Lot #	K1563-4
Purity	>95%
Concentration	0.1 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: M26-11G)

Active HGK (0.1μg/μl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active HGK for optimal results).

Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/μl BSA solution.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250μM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100μl [^{33}P]-ATP (1mCi/100μl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at –20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200μl aliquots at –20°C.

Substrate (Catalog #: M42-51N)

Myelin Basic Protein (MBP) substrate diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [^{33}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active HGK, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.

Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20μl:

- **Component 1.** 10μl of diluted Active HGK (Catalog #M26-11G)
- **Component 2.** 5μl of 1mg/ml stock solution of substrate (Catalog #M42-51N)
- **Component 3.** 5μl distilled H_2O (4°C)

Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H_2O.

Step 5. Initiate the reaction by the addition of 5 μl [^{33}P]-ATP Assay Cocktail bringing the final volume up to 25μl and incubate the mixture in a water bath at 30°C for 15 minutes.

Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20 μl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H_2O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [P^{33}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μl [^{33}P]-ATP / pmoles of ATP (in 5 μl of a 250 μM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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