

KHS1, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # M27-10G

Lot # Q2270-11

Product Description

Recombinant full-length human KHS1 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_006575](#).

Gene Aliases

MAP4K5, KHS, GCKR, MAPK5

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.

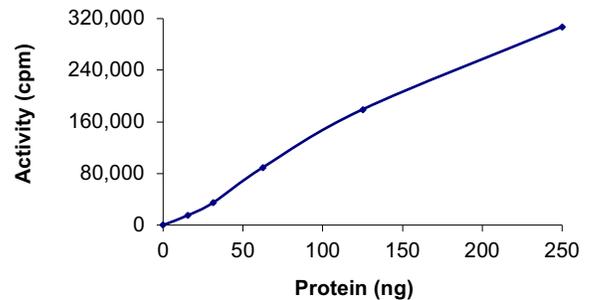
Scientific Background

KHS1 belongs to serine/threonine kinase family that has a STE20-like protein kinase domain which stimulates the stress-activated protein kinase (SAPK, also known as Jun kinase or JNK) pathway (1). It is a mitogen-activated protein kinase kinase kinase kinase 5, termed germinal center kinase related (GCKR). Recently, the KH domain of *Escherichia coli* polynucleotide phosphorylase has been reported to be necessary for autoregulation and growth at low temperature (2).

References

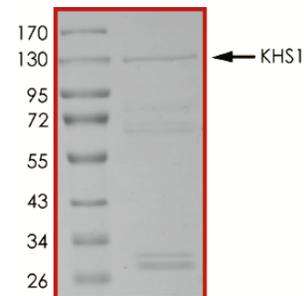
- Shi, C.S. et al: TNF-mediated activation of the stress-activated protein kinase pathway: TNF receptor-associated factor 2 recruits and activates germinal center kinase related. *J. Immunol.* 1999;163(6):3279-85.
- Maura Epifanía, M.O. et al: The KH and S1 domains of *Escherichia coli* polynucleotide phosphorylase are necessary for autoregulation and growth at low temperature. *Biochim. et Biophys. Acta (BBA).* 2007; 1769 (3):194-203

Specific Activity



The specific activity of KHS1 was determined to be **75 nmol /min/mg** as per activity assay protocol.

Purity



The purity of KHS1 was determined to be **>75%** by densitometry.
Observed MW **~125 kDa**.
Calculated MW **~122 kDa**.

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Full-length recombinant protein expressed in Sf9 cells

Catalog #	M27-10G
Specific Activity	75 nmol/min/mg
Lot #	Q2270-11
Purity	>75%
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 #: M27-10G)

Active KHS1 (0.1 µg/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 KHS1 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 MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250µM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³³P]-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) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active KHS1, 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 KHS1 (Catalog #M27-10G)
 - Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #M42-51N)
 - Component 3.** 5µl distilled H₂O (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 H₂O.
- Step 5.** Initiate the reaction by the addition of 5µl [³³P]-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 20µl 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 H₂O) 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 [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [³³P]-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 ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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MATERIAL SAFETY DATA SHEET

Article 1 - Product Identification and Use

Product Name: KHS1, Active

Catalog # M27-10G

This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.

Manufacturer's Name: SignalChem Pharmaceuticals Inc.
Street Address: 110-13120 Vanier Place
City, Prov. Postal Code: Richmond, BC, V6V 2J2
Fax: 604-232-4601
EMERGENCY PHONE: 604-232-4600

Article 2 - Hazardous Ingredients

NOT AVAILABLE. We are not aware of any hazards associated with this product or its ingredients, but the chemical, physical, and toxicological properties of this product have not been investigated thoroughly. Observe normal laboratory precautions.

Article 3 - Physical Data

This product consists of purified protein in Tris-HCl buffer shipped on dry ice. The physical properties of this product have not been investigated thoroughly.

Article 4 - Fire and Explosion Hazard

NOT APPLICABLE

Article 5 - Reactivity Data

NOT APPLICABLE

Article 6 - Toxicologically Data

May be harmful by inhalation, ingestion, or skin absorption. The toxicological properties of this product have not been investigated thoroughly. Exercise due caution.

Article 7 - Preventative Measures

Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.

*****MULTIPLE COMPONENT SPILL OR LEAK PROCEDURES*****

- Wear protective equipment.
 - Absorb on sand or vermiculite and place in closed containers for disposal.
 - Observe all federal, state and local environmental regulations.
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Article 8 - First Aid Measures

- If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
 - In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. If a rash or other irritation develops, call a physician.
 - If inhaled, remove to fresh air. If breathing becomes difficult, call a physician.
 - In case of eye contact, flush with copious amounts of water for at least 15 minutes while separating the eyelids with fingers. Call a physician.
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Article 9 - Preparation

Prepared by: Mya Zhang

Phone#: 1-866-954-6273

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