

KHS1 Kinase

☑ 5 µg



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This product is for *in vitro* research use only and is not intended for use in humans or animals.

Description: Purified recombinant full-length human KHS1 (Met1-Tyr846) kinase, supplied as a GST fusion protein.

Background: KHS1 is a serine/threonine protein kinase that has an STE20-like protein kinase domain and was named as KHS for kinase homologous to SPS1/STE20. KHS1 belongs to the family of group I germinal center kinase (GCK) and is also referred to as GCK-related (GCKR) kinase (1,2). Like other group I GCKs, KHS1 has an amino-terminal kinase domain and a carboxyl-terminal regulatory domain (1). The fact that KHS1 is activated by TNF stimulation and acts as an upstream kinase for stress-activated protein kinase (SAPK, also referred to as Jun kinase or JNK) indicates that KHS1 may have important role in regulating cellular stress response (3,4).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full-length human KHS1 (Met1-Tyr846) (GenBank Accession No. NM_006575) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

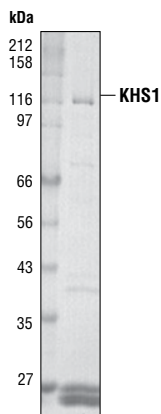


Figure 1. The purity of the KHS1 protein was analyzed using SDS/PAGE followed by Coomassie stain.

Quality Control: The theoretical molecular weight of the KHS1 protein is 120 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. KHS1 kinase activity was determined using a radiometric assay [Fig.2].

Background References:

- (1) Tung, R.M. and Blenis, J. (1997) *Oncogene* 14, 653–9.
- (2) Kyriakis, J.M. (1999) *J Biol Chem* 274, 5259–62.
- (3) Shi, C.S. et al. (2000) *Blood* 95, 776–82.
- (4) Shi, C.S. et al. (1999) *J Immunol* 163, 3279–85.

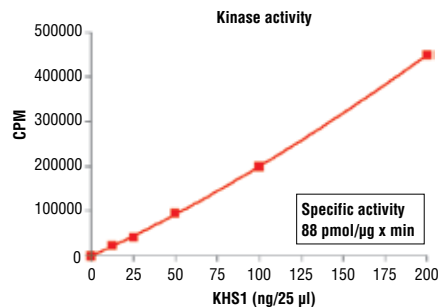


Figure 2. KHS1 kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM MgCl₂, 0.05 mM DTT, 50 µM ATP, Substrate: MBP 200 ng/µL, and variable amount of KHS1.

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH7.5; 150 mM NaCl, 0.25 mM DTT, 0.1mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione.

Store at -80 C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Serine/Threonine Kinase Substrate Screening Kit #7400

Protocol for KHS1 Kinase Assay

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

- 1. Kinase Buffer (5X)**
25 mM MOPS, pH 7.2
12.5 mM β -glycerophosphate
5 mM EGTA
2 mM EDTA
25 mM $MgCl_2$
0.25 mM DTT
- 2.** ATP (10 mM) #9804
- 3.** ^{32}P - γ ATP
- 4.** MBP (0.5 μ g/ μ l)

B Suggested Protocol

- 1.** Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 μ M ATP.
- 2.** Dilute [^{32}P] ATP to 0.16 μ Ci/ μ l [^{32}P] ATP with 250 μ M ATP solution.
- 3.** Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
- 4.** Dilute KHS1 kinase protein (100 ng/ μ l concentration) to 20 ng/ μ l with 1X assay buffer followed by 2-fold serial dilutions.
- 5.** To start the reaction combine 10 μ l diluted KHS1 kinase solution, 10 μ l MBP (0.5 μ g/ μ l), an 5 μ l 0.16 μ Ci/ μ l [^{32}P] ATP solution.
- 6.** After 30 minutes incubation, transfer 5 μ l of this reaction cocktail to a fresh plate and add 10 μ l 1X assay buffer and 5 μ l of MBP (1 μ g/ μ l).
- 7.** Add 5 μ l of the 0.16 μ Ci/ μ l [^{32}P] ATP to start the reaction.

Final Assay Conditions

- 5 mM MOPS, pH 7.2
 - 2.5 mM β -glycerophosphate
 - 1 mM EGTA
 - 5 mM $MgCl_2$
 - 0.05 mM DTT
 - 50 μ M ATP
 - 200 ng/ μ l MBP
- 6.** After 15 minutes terminate reaction by spotting 20 μ l of the reaction mixture onto phosphocellulose P81 paper.
 - 7.** Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
 - 8.** Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
 - 9.** Count samples in a scintillation counter.

Cell Signaling Technology offers a full line of protein kinases, substrates, and antibody detection reagents for high throughput screening. Please direct all inquiries to: drugdiscovery@cellsignal.com.