Store at -80°C

#7448

Hck Kinase

☑5 µg

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New 05/06

Description: Purified recombinant human Hck (Asn230-Arg497) kinase, supplied as a GST fusion protein.

Background: Hck (hemopoietic cell kinase) is a protein tyrosine kinase of the Src family prominently expressed in the lymphoid and myeloid lineages of hemopoiesis (1). It participates in transducing a variety of extracellular signals, which ultimately affect cellular processes including proliferation, differentiation and migration.The well-defined modular structure of Hck comprises a relatively divergent, NH2-terminal "unique" domain, which is subject to post-translational lipid modifications thereby targeting Hck to the plasma membrane. Src homology 3 (SH3) and 2 (SH2) domains, and a tyrosine kinase catalytic domain follow the "unique" domain. The catalytic activity of Hck is regulated, both positively and negatively, by tyrosine phosphorylation of highly conserved tyrosine (Y) residues. Phosphorylation of a single conserved Tyr499 residue in the COOH terminus of Hck by the protein kinase Csk renders Hck inactive as a result of an intramolecular interaction between the phosphorylated tyrosine (pY) residue and its own SH2 domain. Disruption of this interaction, either as a result of dephosphorylation, or substitution of the COOH-terminal regulatory Y residue with phenylalanine (F; e.g., HckY499F), or COOH-terminal truncation mutations as observed in the virally transduced v-Src oncoprotein, results in constitutive activation of Hck. In contrast to phosphorylation of the COOH-terminal regulatory tyrosine residue, autophosphorylation of a tyrosine residue (Tyr388) within the kinase domain of Hck acts to positively regulate its catalytic activity. Thus, activation of Hck requires both disruption of the COOH-terminal regulatory tyrosine-SH2 domain interaction and autophosphorylation of the regulatory tyrosine residue within the kinase domain (2, 3). The dysfunction or dysregulation of Hck may contribute to the pathogenesis of some human leukemias (4).

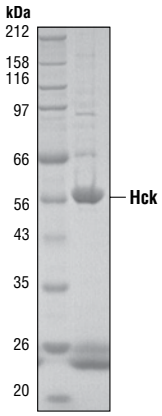

Figure 1. The purity of the GST-Hck fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing human Hck (Asn230-Arg497) (GenBank Accession No. NM_002110) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

Quality Control: The theoretical molecular weight of the GST-Hck fusion protein is 56 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. Hck kinase activity was determined using a radiometric assay [Fig.2].

Background References:

(1) Quintrell, N. et al. (1987) *Mol. Cell. Biol.* 7, 2267–2275.

(2) Ziegler, C.A. et al. (1989) *Mol. Cell. Biol.* 9, 2724–2727.

(3) Kefalas, P. et al. (1995) *Int. J. Biochem. Cell. Biol.* 27, 551–563.

(4) Hu, Y. et al. (2004) *Nat. Genet.* 36, 453–461.

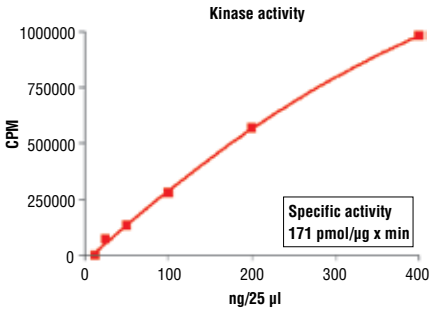

Figure 2. Hck kinase activity was measured in a radiometric assay using the following reaction conditions: 4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM $MgCl_2$, 0.05 mM DTT, 50 µM ATP, Substrate: Poly(Glu-Tyr), 400 ng/µL and recombinant Hck: variable.

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM 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.

Protocol for Hck 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 (10X)**
   40 mM MOPS, pH 7.2
   25 mM β-glycerophosphate
   10 mM EGTA
   4 mM EDTA
   40 mM $MgCl_2$
   100 mM $MnCl_2$
   0.5 mM DTT
2. ATP (10 mM) #9804
3. ^{32}P-γATP
4. Poly(Glu-Tyr) (1 µg/µl)

B Suggested Protocol

1. Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 µM ATP.
2. Dilute [^{32}p] ATP to 0.16 µCi/µl [^{32}p] ATP with 250 µM ATP solution.
3. Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
4. Dilute Hck kinase protein (100 ng/µl concentration) to 20 ng/µl with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 µl diluted Hck kinase solution, 10 µl Poly(Glu-Tyr) (1 µg/µl) and 5 µl 0.16 µCi/µl [^{32}p] ATP solution.

Final Assay Conditions
4 mM MOPS, pH 7.2
2.5 mM β-glycerophosphate
1 mM EGTA
0.4 mM EDTA
4 mM $MgCl_2$
0.05 mM DTT
400 ng/µl Poly(Glu-Tyr)

6. After 15 minutes terminate reaction by spotting 20 µl 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.

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