

Product Specification

HCK, active

(Recombinant human protein, residues 230-497, expressed in Sf 9 cells)

Catalog #: 7720
Lot #: _____
Aliquot size: 5 µg protein in 50 µl
Specific activity: 171 nmol/min/mg

Quality Control Analysis

Activity assessment

HCK protein (100 ng/µl concentration) was diluted to 20ng/µl with assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM MgCl₂, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the poly(Glu-Tyr) in the following assay condition:

- 10 µl Diluted HCK protein
- 10 µl poly(Glu-Tyr) (1 mg/ml stock)
- 5 µl [³²P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [³²P] ATP, were incubated at 30°C and the reaction started by the addition of [³²P] ATP. After 15 minutes, the reaction was terminated by spotting 20 µl of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilutions of the enzyme in the assay, are shown in Fig. 1.

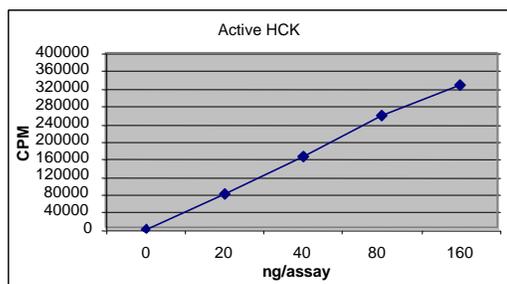


Fig. 1 HCK activity assay

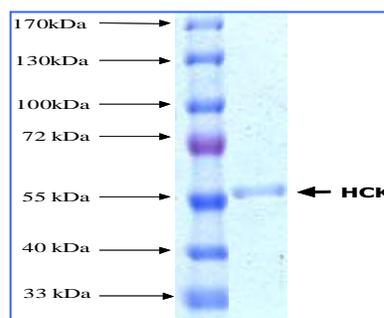


Fig. 2 HCK protein gel

Purity assessment

1 µg of protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the HCK product, and the band was at ~57 kDa (Fig. 2).

Product Description

Recombinant human HCK (230-497aa) containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells. The gene accession number is NM_002110.
This material is sold for research purposes only.

Specific Activity

171 nmol phosphate incorporated into poly(Glu-Tyr) per minute per mg protein at 30° C for 15 minutes using a final concentration of 50 µM ATP (0.83 µCi/assay).

Formulation

Recombinant proteins in storage buffer (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).

Storage and Stability

Store product frozen at or below -70° C. Stable for 1 year at -70° C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

Scientific Background

HCK, a protein-tyrosine kinase, belongs to SRC family members (1). Ziegler et al. found that expression of HCK may be limited to certain hemopoietic cells and is especially prominent in cells of myeloid lineage, particularly mature granulocytes and monocytes (2). Therefore, Quintrell et al. designated the gene HCK (pronounced 'hick') for hemopoietic cell kinase. They described the nucleotide sequence of a cDNA clone and the distribution of RNA transcribed from HCK among various hemopoietic cells. They assigned the HCK gene to 20q11-q12. Since this region is affected by interstitial deletions in some acute myeloid leukemias and myeloproliferative disorders, they suggested that damage to HCK may contribute to the pathogenesis of these conditions (3).

References

1. Piccardoni P, Manarini S, Federico L, Bagoly Z, Pecce R, Martelli N, Piccoli A, Totani L, Cerletti C, Evangelista V. SRC-dependent outside-in signalling is a key step in the process of autoregulation of beta2 integrins in polymorphonuclear cells. *Biochem J.* 2004 May 15;380(Pt 1):57-65.
2. Ziegler, S. F.; Marth, J. D.; Lewis, D. B.; Perlmutter, R. M.: Novel protein-tyrosine kinase gene (hck) preferentially expressed in cells of hematopoietic origin. *Molec. Cell. Biol.* 7: 2276-2285, 1987.
3. Quintrell, N.; Lebo, R.; Varmus, H.; Bishop, J. M.; Pettenati, M. J.; Le Beau, M. M.; Diaz, M. O.; Rowley, J. D.: Identification of a human gene (HCK) that encodes a protein-tyrosine kinase and is expressed in hemopoietic cells. *Molec. Cell. Biol.* 7: 2267-2275, 1987.