

Product Specification

CSK, active

(Full-length recombinant protein expressed in Sf 9 cells)

Catalog #: 7722
Lot #: _____
Aliquot size: 5 µg protein
Specific activity: 425 nmol/min/mg

Quality Control Analysis

Activity assessment

CSK protein (~100 ng/µl concentration) was diluted to 20ng/µl in assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 0.4 mM EDTA, 5 mM MnCl₂, 4 mM MgCl₂, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the substrate Poly(Glu-Tyr) in the assay condition:

- 10 µl Diluted CSK protein
- 5 µl Poly(Glu-Tyr) (1 mg/ml stock)
- 5 µl water
- 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.

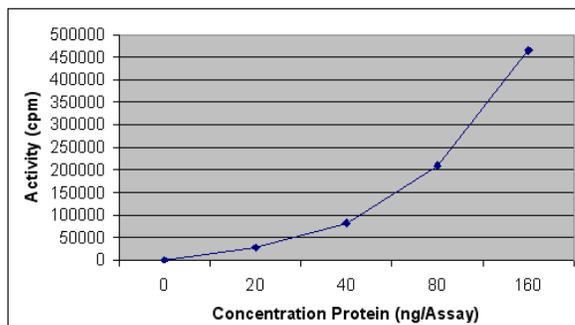


Fig. 1 CSK activity assay

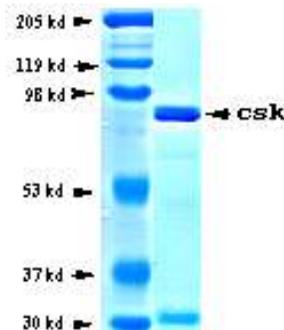


Fig. 2 CSK protein gel

Purity assessment

1.5 µg of CSK protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >85% purity of the CSK product, and the band was at ~78 kDa (Fig. 2).

Product Description

Recombinant full length human CSK containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.

The gene accession number is NM_004383.

This material is sold for research purposes only.

Specific Activity

425 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

Lyophilized from sterile 20 mM Tris, 500 mM NaCl, 0.5 mM PMSF, pH 7.4, 5% trehalose and 5% mannitol.

Reconstitution

Reconstitute in sterile water to a concentration of 0.15 mg/ml.

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

CSK is a cytoplasmic tyrosine kinase that has been shown to downregulate the tyrosine kinase activity of the c-src oncoprotein through tyrosine phosphorylation of the c-src carboxy terminus (1). Cell transformation by src oncoprotein is caused by several oncogenic mechanisms, which interfere with the carboxy terminal phosphorylation. The CSK could therefore potentially function as an anti-oncogene. CSK is ubiquitously expressed in human tissues as 2 mRNA species of 2.6 and 3.4 kb, although in some tissues and cell lines, only the larger mRNA is detected (2). A yeast 2-hybrid system has been used to identify proteins associated with CSK. The Src homology-3 (SH3) domain of CSK associates with a proline-rich region of PEP, a protein-tyrosine phosphatase expressed in hemopoietic cells (2). This association is highly specific and it is speculated that PEP may be an effector and/or regulator of CSK in T cells and other hemopoietic cells. An in situ hybridization has been used to map the CSK gene to chromosome 15q23-q25 (3).

References

1. Partanen, J.; Armstrong, E.; Bergman, M.; Makela, T. P.; Hirvonen, H.; Huebner, K.; Alitalo, K.: Cyl encodes a putative cytoplasmic tyrosine kinase lacking the conserved tyrosine autophosphorylation site (Y416-src). *Oncogene* 6: 2013-2018, 1991.
2. Cloutier, J.-F.; Veillette, A. : Association of inhibitory tyrosine protein kinase p50(csk) with protein tyrosine phosphatase PEP in T cells and other hemopoietic cells. *EMBO J.* 15: 4909-4918, 1996.
3. Armstrong, E.; Cannizzaro, L.; Bergman, M.; Huebner, K.; Alitalo, K.: The c-src tyrosine kinase (CSK) gene, a potential antioncogene, localizes to human chromosome region 15q23-q25. *Cytogenet. Cell Genet.* 60: 119-120, 1992.