CSK, Active
Full-length recombinant protein expressed in Sf9 cells
Catalog # C63-10G
Lot # B049-1

Product Description
Recombinant full-length human CSK was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_004383.

Gene Aliases
None

Formulation
Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 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
CSK is a cytoplasmic tyrosine kinase that has been shown to downregulate the tyrosine kinase activity of the c-src through tyrosine phosphorylation of the c-src carboxy terminus (1). 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.

References

Specific Activity
The specific activity of CSK was determined to be 425 nmol/min/mg as per activity assay protocol.

Purity
The purity was determined to be >85% by densitometry. Approx. MW 78kDa.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: C63-10G)
Active CSK (0.1μg/μl) diluted with Kinase Dilution Buffer IV (Catalog #: K24-09) 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 CSK for optimal results).

Kinase Dilution Buffer IV (Catalog #: K24-09)
Kinase Assay Buffer II (Catalog #: K02-09) diluted at a 1:4 ratio (5X dilution) with 50ng/μl BSA solution.

Kinase Assay Buffer II (Catalog #: K02-09)
Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 20mM MgCl2, 25mM MnCl2, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[32P]-ATP Assay Cocktail
Prepare 250μM [32P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100μl [32P]-ATP (1mCi/100μl), 5.75ml of Kinase Assay Buffer II (Catalog #: K02-09). 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 II (Catalog #: K02-09). Store 200μl aliquots at -20°C.

Substrate
Poly (Glu:Tyr, 4:1) synthetic peptide substrate diluted in distilled H2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [32P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
Step 2. Thaw the Active CSK, 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 CSK (Catalog #C63-10G)
   Component 2. 10μl of 1mg/ml stock solution of substrate
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 [32P]-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 [P32]-ATP Specific Activity (SA) (cpm/pmol)
Specific activity (SA) = cpm for 5μl [32P]-ATP / pmoles of ATP (in 5μl of a 250μM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)
Corrected cpm from reaction / ([SA of 32P-ATP in cpm/pmol] * (Reaction time in min) * (Enzyme amount in μg or mg)) / ([Reaction Volume] / (Spot Volume))