p70S6K, Active
Full-length recombinant protein expressed in Sf9 cells
Catalog # R21-10H
Lot # N114-3

Product Description
Recombinant full-length human p70S6K was expressed by baculovirus in Sf9 insect cells using an N-terminal His tag. The gene accession number is NM_003161.

Gene Aliases
S6K1; STK14A RPS6KB1

Formulation
Recombinant protein stored in 50mM sodium phosphate, pH 7.0, 300mM NaCl, 150mM imidazole, 0.1mM PMSF, 0.25mM DTT, 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
p70S6K is responsible for the phosphorylation of 40S ribosomal protein S6 and is ubiquitously expressed in human adult tissues (1). p70S6K is activated by serum stimulation and this activation is inhibited by wortmannin and rapamycin. p70S6K activity undergoes changes in the cell cycle and increases 20-fold in G1 cells released from G0 (2). p70S6K activation requires sequential phosphorylations at proline-directed residues in the putative autoinhibitory pseudosubstrate domain, as well as threonine 389, a site phosphorylated by phosphoinositide-dependent kinase 1 (PDK-1).

References

Specific Activity
The specific activity of p70S6K was determined to be 89 nmol/min/mg as per activity assay protocol.

Purity
The purity of p70S6K was determined to be >85% by densitometry. Approx. MW 76kDa.

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Activity Assay Protocol

Active Kinase (Catalog #: R21-10H)
Active p70S6K (0.1µg/µl) diluted with Kinase Dilution Buffer I (Catalog #: K21-09) 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 p70S6K for optimal results).

Kinase Dilution Buffer I (Catalog #: K21-09)
Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with distilled H2O.

Kinase Assay Buffer I (Catalog #: K01-09)
Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM MgCl2, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[33P]-ATP Assay Cocktail
Prepare 250µM [33P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [33P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). 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 I (Catalog #: K01-09). Store 200µl aliquots at –20°C.

Substrate (Catalog #: S05-58)
S6K synthetic peptide substrate (KRRRLASLR) diluted in distilled H2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [33P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active p70S6K, 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 p70S6K (Catalog #R21-10H)
- Component 2. 5µl of 1mg/ml stock solution of substrate (Catalog #S05-58)
- Component 3. 5µl distilled H2O (4°C)

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 [33P]-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 (cpm) 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 [P³³]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [³³P]-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 ³³P-ATP in cpm/pmol]*[Reaction time in min]*[Enzyme amount in µg or mg]) / ([Reaction Volume] / [Spot Volume])

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