Recombinant Human Active p70 S6 Kinase Protein, CF

Catalog Number: 896-KS-010
Lot Number: 1757181

SPECIFICATIONS AND USE

Source	Recombinant full-length human p70 S6 Kinase (p70 S6K) was expressed by baculovirus in *Sf*9 insect cells using an N-terminal His tag. The gene accession number is NM_003161.
Molecular Mass	The approximate molecular weight is 76 kDa.
Purity	The purity was determined to be > 85% by densitometry.
Formulation	Supplied in 50 mM Sodium Phosphate (pH 7.0), 300 mM NaCl, 0.25 mM DTT, 150 mM Imidazole, 0.1 mM PMSF, and 25% Glycerol.
Size	10 µg
Concentration	0.1 µg/µL
Activity	The specific activity of p70 S6K was determined to be 73 nmol/min/mg using a synthetic peptide substrate.
Storage	This product is stable at ≤ -70 °C for up to one year from the date of receipt. For optimal storage, aliquot into smaller quantities after centrifugation and store at recommended temperature. **Avoid repeated freeze-thaw cycles.**

BACKGROUND

p70 S6K 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 T389, a site phosphorylated by phosphoinositide-dependent kinase 1 (PDK-1).

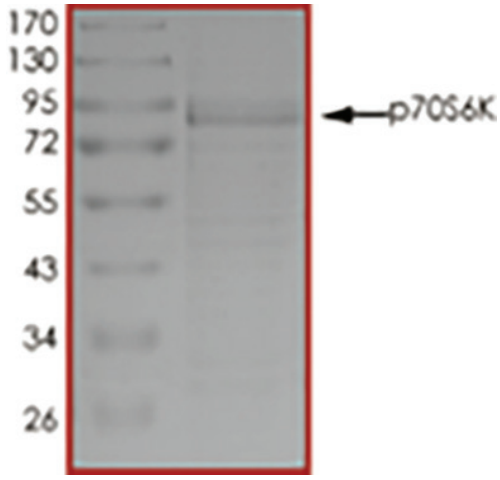

Figure 1: The approximate molecular weight is 76 kDa and the purity is > 85%.

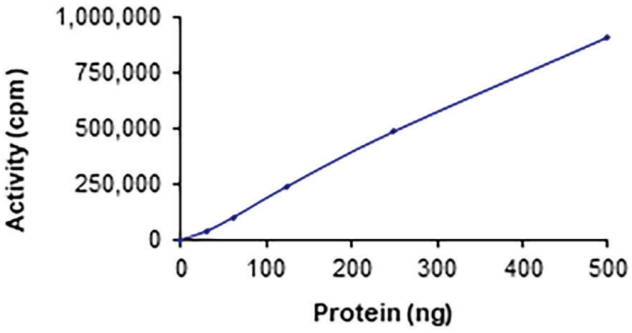

Figure 2: The specific activity of p70 S6K was determined to be 73 nmol /min/mg as per Activity Assay Protocol.

REFERENCES

1. Ferrari, S. *et al*. (1994) Crit. Rev. Biochem. Mol. Biol. **29**:385.
2. Edelmann, H.M. *et al*. (1996) J. Biol. Chem. **271**:963.

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ACTIVITY ASSAY PROTOCOL

Solutions Required

Active Kinase - Active p70 S6K (0.1 µg/µL) diluted with Kinase Dilution Buffer I 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 p70 S6K for optimal results.*

Kinase Dilution Buffer I - Kinase Assay Buffer I diluted at a 1:4 ratio (5X dilution) with distilled water.

Kinase Assay Buffer I - 25 mM MOPS pH 7.2, 12.5 mM β-glycerolphosphate, 25 mM $MgCl_2$, 5 mM EGTA, 2 mM EDTA. Add 0.25 mM DTT to the Kinase Assay Buffer I prior to use.

[^{33}P]-ATP Assay Cocktail - Prepare 250 µM [^{33}P]-ATP Assay Cocktail in a designated radioactive work area by combining 150 µL of 10 mM ATP Stock Solution, 100 µL of [^{33}P]-ATP (1 mCi/100 µL), and 5.75 mL of Kinase Assay Buffer I. Store 1 mL aliquots at ≤ -20 °C.

10 mM ATP Stock Solution - Prepare the ATP Stock Solution by dissolving 55 mg of ATP in 10 mL of Kinase Assay Buffer I. Store 200 µL aliquots at ≤ -20 °C.

Substrate - S6K synthetic peptide substrate (KRRRLASLR) diluted in distilled water to a final concentration of 1 mg/mL.

ASSAY PROCEDURE

1. Thaw the [^{33}P]-ATP Assay Cocktail in a shielded container in a designated radioactive work area.
2. Thaw the Active p70S6K, Kinase Assay Buffer I, Substrate, and Kinase Dilution Buffer I on ice.
3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 µL.

Reaction Component	Amount
Diluted Active p70S6K	10 µL
Substrate (1 mg/mL Stock Solution)	5 µL
Distilled water (2-8 °C)	5 µL

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 or deionized water.
5. Initiate the reaction by the addition of 5 µL [^{33}P]-ATP Assay Cocktail, bringing the final volume up to 25 µL. Incubate the mixture in a water bath at 30 °C for 15 minutes.
6. After the 15 minute incubation period, terminate the reaction by spotting 20 µL of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (add 10 mL of phosphoric acid to 990 mL of distilled water) with constant gentle stirring. It is recommended that the strips be washed a total of three times for approximately 10 minutes each.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 4) for each sample and calculate the Kinase Specific Activity as outlined below.

Calculation of [^{33}P]-ATP Specific Activity (SA) (cpm/pmol)

Specific Activity (SA) = cpm for 5 µL [^{33}P]-ATP/pmole of ATP (in 5 µL of a 250 µM ATP stock solution, i.e. 1250 pmoles)

Calculation of Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol) x (Reaction time in minutes) x (Enzyme amount in µg or mg)] x [(Reaction volume) / (Spot Volume)]