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Recombinant full-length human p70S6K kinase containing N-terminal His tag

Catalog No.	CRP104A	**Quantity:**	5 µg
	CRP104B		20 µg

Description: Recombinant full-length human p70S6K containing N-terminal His tag was expressed by baculovirus in Sf 9 insect cells.

Activation of cell growth leads to the multiple phosphorylation of 40S ribosomal protein S6. The kinase responsible for controlling this event is termed p70S6K. Northern blot analysis shows p70S6K to be ubiquitously expressed in human adult tissues. p70S6K is activated by serum stimulation, and the serum-induced 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. The kinase is reactivated 10-fold when cells released from a nocodazole-induced metaphase block enter G1 of the next cell cycle. The immunosuppressive agent rapamycin induces inactivation of p70s6k with no effect on other mitogen-activated kinases. The principal target of rapamycin-induced p70s6k inactivation is T389 site on p70S6K, which is located in an unusual hydrophobic sequence outside the catalytic domain. Mutation of T389 to alanine ablates kinase activity, whereas mutation to glutamic acid confers constitutive kinase activity and rapamycin resistance. p70S6K activation by growth factor requires phosphorylation by various inputs on multiple sites. p70S6K activation requires sequential phosphorylations at proline-directed residues in the putative autoinhibitory pseudosubstrate domain, as well as threonine 389. Threonine 229, a site in the catalytic loop is phosphorylated by PDK-1. Activation of p70S6K requires a phosphoinositide 3-kinase (PI3-K)-dependent signal(s).

Concentration: 0.1 mg/ml

Gene Accession No: NM_003161

Source: Sf9 insect cells

Formulation: Recombinant protein in storage buffer (50 mM Tris-HCl + 150 mM NaCl + 0.25 mM DTT + 0.1 mM EGTA + 0.1 mM EDTA + 0.1 mM PMSF + 25% glycerol; pH 7.5).

Purity: 1 µg of p70S6K protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the p70S6K product, and the band was at ~76 kDa (Fig. 2).

Specific Activity: 80 nmol/min/mg: 80 nmol phosphate incorporated into S6K substrate peptide per minute per mg protein at 30ºC for 15 minutes using a final concentration of 50 µM ATP (0.83 µCi/assay). See QA/QC section for details.

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Storage & Stability: Store product frozen at or below -70ºC. Stable for 1 year at -70ºC as undiluted stock. Aliquot to avoid repeated thawing and freezing.

QA/QC: p70S6K protein (100 ng/µl concentration) was diluted to 50 ng/µl with assay dilution buffer (4 mM MOPS + 2.5 mM β-glycerophosphate + 1 mM EGTA + 0.4 mM EDTA + 4 mM $MgCl_2$ + 0.05 mM DTT; pH 7.2), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the S6K substrate peptide (CKRRRLASLR) in the following assay condition:

- 10 µl diluted p70S6K protein
- 10 µl S6K substrate peptide (1 mg/ml stock)
- 5 µl [^{32}P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [^{32}P] ATP, were incubated at 30°C and the reaction started by the addition of [^{32}P] 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 p70S6K activity assay

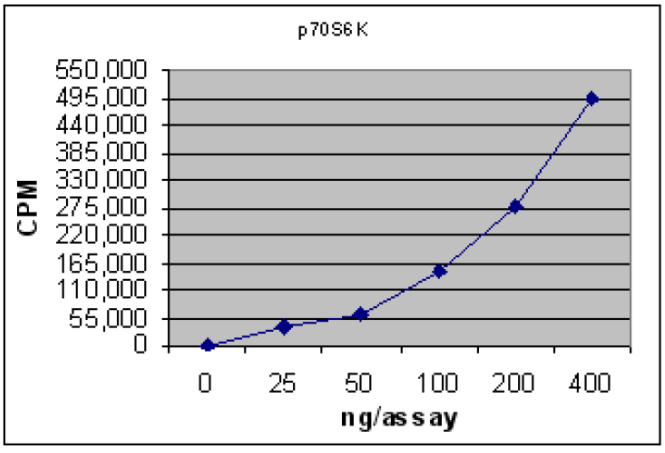
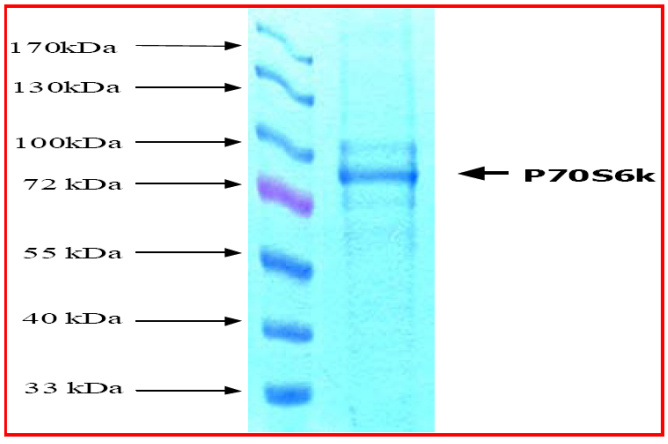


Fig. 2 p70S6K protein gel



Please note: always centrifuge vials before opening.

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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