# p70S6K (Human), Active

## Full-length recombinant protein expressed in Sf9 cells

## Cat# CY-SPR21

**Lot No. K102-2**
**5 µg 0.1 µg/µl**

**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).

**Product Description:**

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

**Gene Aliases:**

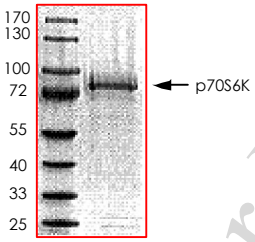
S6K1; STK14A RPS6KB1

**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.

**Purity & Molecular Weight:**

The purity was determined to be >80% by densitometry. Approx. MW 76kDa.




**Storage:**

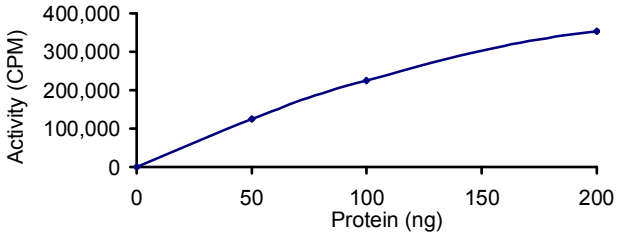
Store product at –70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

**Stability:**

Unopened vial at –70 °C, 1 year from date of shipment.

**Specific Activity:**

The specific activity was determined to be 78 nmol/min/mg as per Activity Assay Protocol.




**Activity Assay Protocol:**

Assay activity of the kinase in a 25 µL reaction consisting of 5 µL of 5 X Kinase Assay Buffer, 10 µL of 1 mg/ml the Substrate Solution, 5 µL of diluted kinase and 5 µL of 250 µM ATP solution containing [gamma $^{32}$P] ATP (0.167 µCi/µL). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20 µL of the reaction mixture onto phosphocellulose P81 paper. Air-dry the P81 paper and sequentially wash 4 times for approximately 10 minutes each in 1% phosphoric acid with constant gentle stirring. Count the P81 paper in a liquid scintillation counter.

**Substrate Solution:**

S6K synthetic peptide substrate (CKRRRLASLR) diluted in distilled $H_2O$ to a final concentration of 1 mg/ml.

**5 X Kinase Assay Buffer:**

25mM MOPS, pH 7. 2, 12.5mM $\beta$ -glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

**References:**

1.Ferrari, S. et al: S6 phosphorylation and the p70s6k/p85s6k. Crit Rev Biochem Mol Biol. 1994;29(6):385-413. Review.

2.Edelmann, HM. Et al: Cell cycle regulation of p70 S6 kinase and p42/p44 mitogen-activated protein kinases in Swiss mouse 3T3 fibroblasts. J Biol Chem. 1996 Jan 12;271(2):963-71.

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