BioVision
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RSK2, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # 7768-5
Lot# _____

Aliquot Size:	5 µg in 50 µl/vial
Concentration:	0.1 µg/µl
Purity:	>90%
Storage:	-80°C
Shipping:	in Dry ice
Shelf Life:	6-12 months from shipping date
Specific Activity:	157 nmol/min/mg

Product Description

Recombinant full-length human RSK2 was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is NM_004586.

Gene Aliases

RPS6KA3; HU-3; MAPKAPK1B; CLS; MRX19; ISPK-1; p90-RSK2; pp90RSK2; S6K-alpha3

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.

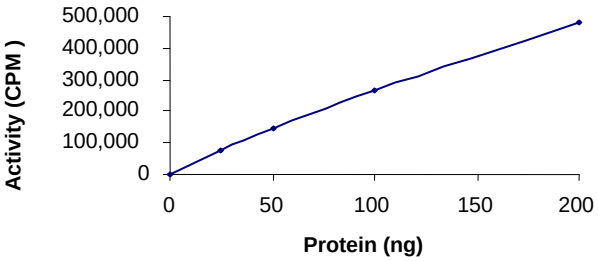
Scientific Background

RSK2 is a member of the RSK (ribosomal S6 kinase) family that are growth factor-regulated serine/threonine kinases. RSK2 has been shown to mediate growth factor signaling via RAS and MAPK leading to the induction of CREB serine-133 phosphorylation and activation of gene expression (1). Mutations in RSK2 have been shown to be responsible for Coffin-Lowry syndrome (CLS) which is a X-linked disorder characterized by severe psychomotor retardation, facial and digital dysmorphisms, and progressive skeletal deformations (2).

References

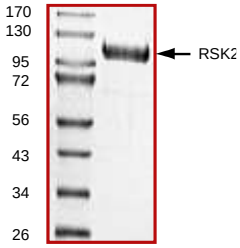
1. Xing, J. et al: Coupling of the RAS-MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase. Science. 1996 Aug 16;273(5277):959-63.
2. Jacquot, S. et al: Mutation analysis of the RSK2 gene in Coffin-Lowry patients: extensive allelic heterogeneity and a high rate of de novo mutations. Am J Hum Genet. 1998 Dec;63(6):1631-40

Specific Activity



The specific activity of RSK2 was determined to be **157 nmol/min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>90%** by densitometry.
Approx. MW **112kDa**.

Activity Assay Protocol

Reaction Components

Active Kinase 7768-5

Active RSK1 (0.1μg/μl) diluted with Kinase Dilution Buffer 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 RSK1 for optimal results).

Kinase Dilution Buffer

Kinase Assay Buffer diluted at a 1:4 ratio (5X dilution) with distilled H_2O.

Kinase Assay Buffer

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

[^{32}P]-ATP Assay Cocktail

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

10mM ATP Stock Solution

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer. Store 200μl aliquots at –20°C.

Substrate

RSK synthetic peptide substrate (KRRRLSSLRA) diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [^{32}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active RSK2, Kinase Assay Buffer, Substrate and Enzyme 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 RSK2.
- **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 H_2O.

Step 5. Initiate the reaction by the addition of 5µl [^{32}P]-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 H_2O) 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 [P^{32}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [^{32}P]-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 ^{32}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]