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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
R17-10G -05	5 µg
R17-10G -10	10 µg

RSK2, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # R17-10G

Lot # S156-3

Product Description

Recombinant full-length human RSK2 was expressed by baculovirus in Sf9 insect cells using an 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, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, and 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.

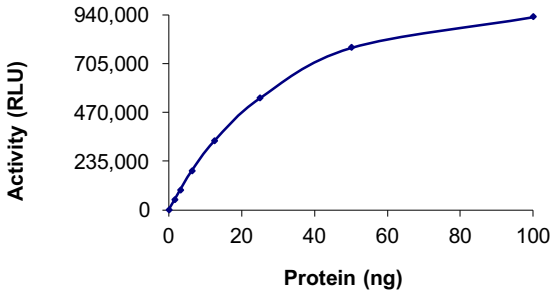
Scientific Background

RSK2 is a member of the RSK (ribosomal S6 kinase) family that consists of 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 an X-linked disorder characterized by severe psychomotor retardation, facial and digital dysmorphisms, and progressive skeletal deformations (2).

References

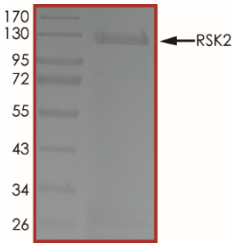
1. Xing, J. et al: Coupling of the RAS-MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase. Science. 1996 Aug 16;273(5277):959-63.
2. Jacquot, S. et al: Mutation analysis of the RSK2 gene in Coffin-Lowry patients: extensive allelic heterogeneity and a high rate of de novo mutations.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

Specific Activity



The specific activity of RSK2 was determined to be **110 nmol /min/mg** as per activity assay protocol, and was equivalent to **157 nmol/min/mg** as per radiometric assay.

Purity



The purity of RSK2 was determined to be **>90%** by densitometry. RSK2 Approx. MW **112kDa**.

RSK2, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog #	R17-10G
Specific Activity	110 nmol/min/mg
Lot #	S156-3
Purity	>90%
Concentration	0.1 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	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. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: R17-10G)

Active RSK2 (0.1μg/μl) diluted with Kinase Dilution Buffer X (1x) (Catalog #: K20-09) 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 RSK2 for optimal results).

Kinase Assay Buffer III (5x) (Catalog #: K03-09)

Buffer components: 200mM Tris-HCl, pH 7.4, 100mM $MgCl_2$ and 0.5mg/ml BSA. Add fresh DTT prior to use to a final concentration of 250μM.

Kinase Dilution Buffer IX (1x) (Catalog #: K29-09)

Kinase Assay Buffer III (Catalog #: K03-09) diluted at a 1:4 ratio (5X dilution) with cold water. Add fresh DTT to the aliquot prior to use to a final concentration of 50μM.

ADP-GloTM Kinase Assay Kit (Promega, Cat # V9101)

ATP solution, 10 mM
ADP solution, 10 mM
ADP-Glo™ Reagent
Kinase Detection Reagent

Substrate (Catalog #: S06-58)

RSK synthetic peptide substrate (KRRRLSSLRA) diluted in distilled H_2O to a final concentration of 0.2mg/ml.

Assay Protocol

The RSK2 assay is performed using the ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the RSK2 reaction. The ADP- Glo™ Reagent is added to terminate the kinase reaction and to deplete the remaining ATP, and then the Kinase Detection Reagent is added to convert ADP to ATP and to measure the newly synthesized ATP using luciferase/luciferin reaction.

Step 1. Thaw the Active RSK2, Kinase Assay Buffer III (5x), and Substrate on ice. Prepare a 15 μL enzyme dilution at the desired concentration, with Kinase Dilution Buffer IX (1x), in a pre-chilled 96-well plate.

Step 2. Prepare a substrate/ATP mixture as follows (25 μM example):

Component	Amount (μL)	Component	Amount (μL)
10μM ATP Solution	1	Substrate at 1mg/mL	80
Kinase Assay Buffer III (5x)	79		

Step 3. Transfer the following reaction components prepared in Step 2 to a 384-well opaque plate bringing the reaction volume up to 5μL:

Component 1. 3μl of diluted Active RSK2 (Catalog # R17-10G).
Component 2. 2μl of Substrate/ATP mix as prepared in the table above. This initiates the reaction.

Step 4. Set up the blank control as outlined in step 2, excluding the addition of the kinase. Replace the kinase with an equal volume of Kinase Dilution Buffer IX (1x).

Step 5. Incubate at ambient temperature for 40 minutes.

Step 6. After the 40-minute incubation period, terminate the reaction and deplete the remaining ATP by adding 5μl of ADP-Glo™ Reagent. Spin down and shake the 384-well plate. Then incubate the reaction mixture for another 40 minutes at ambient temperature.

Step 7. Then add 10μl of the Kinase Detection Reagent to the 384-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.

Step 8. Read the 384-well reaction plate using the Luminescence Module Protocol on a GloMax®-Multi Microplate Multimode Reader (Promega; Cat# E7061).

Step 9. Determine the corrected activity (RLU) by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of Specific Activity of ADP (RLU/pmol)

From ADP standard curve, determine RLU/pmol of ADP

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

Corrected RLU from reaction / [(SA of ADP in RLU/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)

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