BioVision
BioVision Incorporated
980 Linda Vista Avenue Mountain View, CA 94043 USA Phone: (650)428-0236 Fax: (650)428-0336

Product Specification

RSK3, Active

Full-length recombinant protein expressed in Sf9 cells

Cat.#	7774-5
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:	131 nmol/min/mg

Product Description

Recombinant full-length human RSK3 was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is NM_021135.

Gene Aliases

RPS6KA2; HU-2, MAPKAPK1C, S6K-alpha, S6K-alpha2, p90-RSK3, pp90RSK3

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

RSK3 is a member of the RSK (ribosomal S6 kinase) family

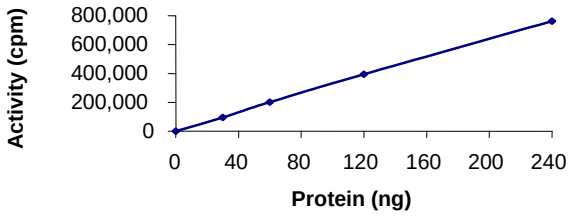
Upon stimulation, RSK3 translocates to the cell nucleus and phosphorylates nuclear proteins. RSK3 can bind to ERK1/2 and this association increases the duration of RSK3 activation (2).

References

1. Zhao, Y. et al: RSK3 encodes a novel pp90rsk isoform with a unique N-terminal sequence: growth factor-stimulated kinase function and nuclear translocation. Mol Cell Biol. 1995 Aug;15(8):4353-63.

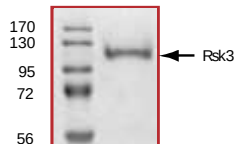
2. Roux, P P. et al: Phosphorylation of p90 ribosomal S6 kinase (RSK) regulates extracellular signal-regulated kinase docking and RSK activity. Mol Cell Biol. 2003 Jul;23(14):4796-804.

Specific Activity



The specific activity of RSK3 was determined to be **131 nmol/min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>90%** by densitometry.

Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: 7774-5)

Active RSK3 (0.1μg/μl) diluted with Kinase Dilution Buffer (see below for details) 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).

[^{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.

Kinase Dilution Buffer

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

10mM ATP Stock Solution

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

Kinase Assay Buffer I

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.

Substrate

RSK synthetic peptide substrate (KRRRLSSLRA) diluted in distilled H_2O to a final concentration of 1 mg/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 RSK3, 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.** 10 μl of diluted Active RSK3
- **Component 2.** 10 μl of 1 mg/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 20 μl 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 10 ml of phosphoric acid and make a 1 L 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.

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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)]