

## RSK2, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # 7768-5

Lot# \_\_\_\_\_

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

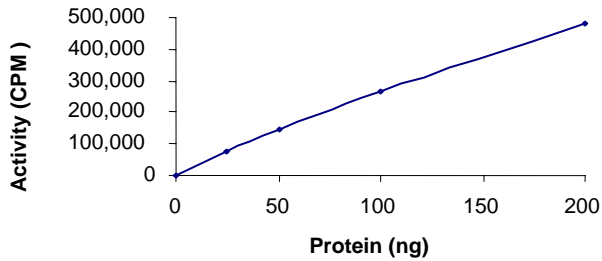
### Scientific Background

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

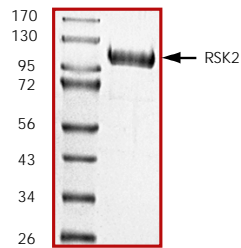
1. Xing, J. et al: Coupling of the RAS-MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase. Science. 1996 Aug 16;273(5277):959-63.
2. Jacquot, S. et al: Mutation analysis of the RSK2 gene in Coffin-Lowry patients: extensive allelic heterogeneity and a high rate of de novo mutations. 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 (0.1 $\mu$ g/ $\mu$ l) 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<sub>2</sub>O.

### Kinase Assay Buffer

Buffer components: 25mM MOPS, pH 7.2, 12.5mM  $\beta$ -glycerol-phosphate, 25mM MgCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [<sup>32</sup>P]-ATP Assay Cocktail

Prepare 250 $\mu$ M [<sup>32</sup>P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 $\mu$ l of 10mM ATP Stock Solution, 100 $\mu$ l [<sup>32</sup>P]-ATP (1mCi/100 $\mu$ l), 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 200 $\mu$ l aliquots at -20°C.

### Substrate

RSK synthetic peptide substrate (KRRRLSSLRA) diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml.

## Assay Protocol

- Step 1.** Thaw [<sup>32</sup>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 20 $\mu$ l:
  - Component 1.** 10 $\mu$ l of diluted Active RSK2.
  - Component 2.** 10 $\mu$ l 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<sub>2</sub>O.

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- Step 5.** Initiate the reaction by the addition of 5 $\mu$ l [<sup>32</sup>P]-ATP Assay Cocktail bringing the final volume up to 25 $\mu$ l 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 $\mu$ l 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<sub>2</sub>O) 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<sup>32</sup>]-ATP Specific Activity (SA) (cpm/pmol)**

Specific activity (SA) = cpm for 5 $\mu$ l [<sup>32</sup>P]-ATP / pmoles of ATP (in 5 $\mu$ l of a 250 $\mu$ M ATP stock solution, i.e., 1250 pmoles)

**Kinase Specific Activity (SA) (pmol/min/ $\mu$ g or nmol/min/mg)**

Corrected cpm from reaction / [(SA of <sup>32</sup>P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in  $\mu$ g or mg)]\*[(Reaction Volume) / (Spot Volume)]