

# RSK2 Kinase

✓ 5 µg



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This product is for *in vitro* research use only and is not intended for use in humans or animals.

**Description:** Purified recombinant full length human RSK2 (Met1-Leu740) kinase, supplied as a GST fusion protein.

**Background:** The 90 kDa ribosomal S6 kinases (RSK1-3) are a family of broadly expressed serine/threonine kinases activated by extracellular signal-regulated protein kinases (Erk1 and 2) in response to many growth factors, polypeptide hormones and neurotransmitters (1). p90RSK is activated by MAPK *in vitro* and *in vivo* via phosphorylation (4). Several sites, such as Ser380, Thr359, Ser363 and Thr573 are important for its activation (3). RSK3 resembles p90RSK by having two kinase domains connected by a regulator linker region (2). Phosphorylation of p90RSK (and likely RSK2 and RSK3) in the linker region is critical for its activity (3).

**Source/Purification:** The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full length human RSK2 (Met1-Leu740) (GenBank Accession No. NM\_004586) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using GSH-agarose.

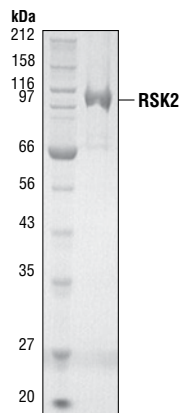


Figure 1. The purity of the GST-RSK2 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

**Quality Control:** The theoretical molecular weight of the GST-RSK2 fusion protein is 112 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. RSK2 kinase activity was determined using a radiometric assay [Fig.2].

#### Background References:

- (1) Frodin, M. and Gammeltoft, S. (1999) *Mol. Cell. Endocrinol.* 151, 65–77.
- (2) Pierrat, B. et al. (1998) *J. Biol. Chem.* 273, 29661–29671.
- (3) Dalby, K. et al. (1998) *J. Biol. Chem.* 273, 1496–1505.
- (4) Lazar, D.F. et al. (1995) *J. Biol. Chem.* 270, 20801–20807.

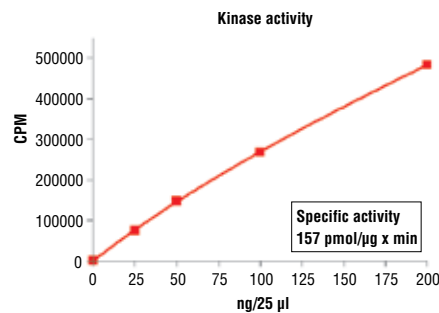


Figure 2. RSK2 kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 5 mM EGTA, 0.4 mM EDTA, 5 mM MgCl<sub>2</sub>, 0.05 mM DTT, 50 µM ATP. Substrate: S6K synthetic peptide substrate, 400 ng/µL, and recombinant RSK2: variable.

**Storage:** Enzyme is supplied in 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione. Store at -80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

#### Companion Products:

Serine/Threonine Kinase Substrate Screening Kit #7400

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

## Protocol for RSK2 Kinase Assay

**Note:** Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

### A Additional Solutions and Reagents (Not included)

#### 1. Kinase Buffer (10X)

50 mM MOPS, pH 7.2  
25 mM  $\beta$ -glycerophosphate  
10 mM EGTA  
4 mM EDTA  
50 mM  $MgCl_2$   
0.5 mM DTT

#### 2. ATP (10 mM) #9804

#### 3. $^{32}P$ - $\gamma$ ATP

#### 4. S6K synthetic peptide substrate (KRRRLASLR) (1 mg/ml)

### B Suggested Protocol

1. Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250  $\mu$ M ATP.
2. Dilute [ $^{32}P$ ] ATP to 0.16  $\mu$ Ci/ $\mu$ l [ $^{32}P$ ] ATP with 250  $\mu$ M ATP solution.
3. Transfer enzyme from  $-80^\circ C$  to ice. Allow enzyme to thaw on ice.
4. Dilute RSK2 protein (100 ng/ $\mu$ l concentration) to 20 ng/ $\mu$ l with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10  $\mu$ l diluted RSK2 kinase solution, 10  $\mu$ l S6K synthetic peptide substrate (KRRRLASLR) (1 mg/ml), and 5  $\mu$ l 0.16  $\mu$ Ci/ $\mu$ l [ $^{32}P$ ] ATP solution.

### Final Assay Conditions

- 5 mM MOPS, pH 7.2  
2.5 mM  $\beta$ -glycerophosphate  
1 mM EGTA  
0.4 mM EDTA  
5 mM  $MgCl_2$   
0.05 mM DTT  
400 ng/ $\mu$ l S6K synthetic peptide substrate (KRRRLASLR)
6. After 15 minutes terminate reaction by spotting 20  $\mu$ l of the reaction mixture onto phosphocellulose P81 paper.
  7. Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
  8. Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
  9. Count samples in a scintillation counter.

Cell Signaling Technology offers a full line of protein kinases, substrates, and antibody detection reagents for high throughput screening. Please direct all inquiries to: [drugdiscovery@cellsignal.com](mailto:drugdiscovery@cellsignal.com).