

Product Specification

RSK1, active

(Full-length recombinant protein expressed in Sf 9 cells)

Catalog #: 7721
Lot #: _____
Aliquot size: 5 µg protein in 50 µl
Specific activity: 38 nmol/min/mg

Quality Control Analysis

Activity assessment

RSK1 protein (~100 ng/µl concentration) was diluted to 20ng/µl with assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM MgCl₂, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the S6K substrate peptide (CKRRRLASLR) in the following assay condition:

10 µl diluted RSK1 protein
10 µl S6K substrate peptide
5 µl [³²P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [³²P] ATP, were incubated at 30°C and the reaction started by the addition of [³²P] ATP. After 15 minutes, the reaction was terminated by spotting 20 µl of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilutions of the enzyme in the assay, are shown in Fig. 1.

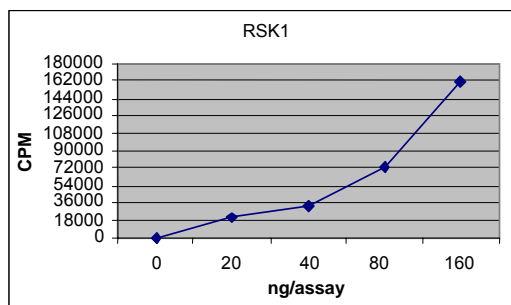


Fig. 1 RSK1 activity assay

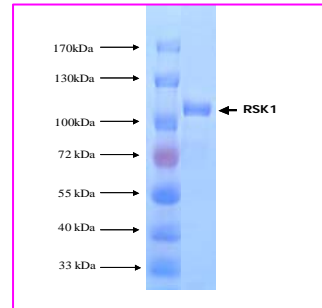


Fig. 2 RSK1 protein gel

Purity assessment

1 µg of RSK1 protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the RSK1 product, and the band was at ~108 kDa (Fig. 2).

Product Description

Recombinant full-length human RSK1 containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells. The gene accession number is NM_002953.

This material is sold for research purposes only.

Specific Activity

38 nmol phosphate incorporated into S6K substrate peptide minute per mg protein at 30°C for 15 minutes using a final concentration of 50 μ M ATP (0.83 μ Ci/assay).

Formulation

Recombinant protein in storage buffer (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).

Storage and Stability

Store product frozen at or below -70°C. Stable for 1 year at -70°C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

Scientific Background

The RSK (ribosomal S6 kinase) family comprises growth factor-regulated serine/threonine kinases, known also as p90(rsk). RSK1 contains 2 nonidentical kinase catalytic domains and phosphorylates various substrates, including members of the mitogen-activated kinase (MAPK) signalling pathway. Moller described the cloning and characterization of 3 genes encoding RSKs, and HU1 (also named RPS6KA1, or RSK1) cDNA encodes a predicted 735-amino acid protein containing 2 distinct consensus ATP-binding site sequences. Northern blot and RNase protection analyses detected an approximately 3.5-kb HU1 transcript in lymphocytes, skeletal muscle, liver, and adipose tissue (1). Zeniou determined the expression of the RSK1, RSK2, and RSK3 genes in various human tissues, during mouse embryogenesis, and in mouse brain (2). RSKs are implicated in the activation of the mitogen-activated kinase (MAPK) cascade and the stimulation of cell proliferation and differentiation (3).

References

1. Moller, D.E.; Xia, C.H.; Tang, W.; Zhu, A.X.; Jakubowski, M.: Human rsk isoforms: cloning and characterization of tissue-specific expression. *Am. J. Physiol.* 266: C351-C359, 1994.
2. Zeniou, M.; Ding, T.; Trivier, E.; Hanauer, A.: Expression analysis of RSK gene family members: the RSK2 gene, mutated in Coffin-Lowry syndrome, is prominently expressed in brain structures essential for cognitive function and learning. *Hum. Molec. Genet.* 11: 2929-2940, 2002.
3. Gross, S.D.; Schwab, M.S.; Lewellyn, A.L.; Maller, J.L.: Induction of metaphase arrest in cleaving *Xenopus* embryos by the protein kinase p90(Rsk). *Science* 286: 1365-1367, 1999.