

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of RSK4 [1 - 745]

Enzyme description:- RSK4 [1 - 745]

Clone number:- DU 63293

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH-Sepharose

Calculated molecular mass:-

Monoisotopic 110, 625.15 daltons

Average Mass 110, 696.21 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.88

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay Buffer:-

50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 10 mM DTT, 10 mM MgAc

Substrate:-

Long S6 [KEAKEKRQEIQIAKRRRLSSLRASTSKSGGSQK]

Final concentration: 30 µM

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Clone Data Sheet

RSK4 [1 - 745]

<u>Protein</u>	RSK4 [1 - 745]
<u>Clone number</u>	DU 63293
<u>Species</u>	Human
<u>Accession number</u>	NM_014496.5
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL FQGPLGSMLPFAPQDEPWDRE MEVFSGGGASSGEVNLK MVDEPMEEGEADSCHDEGVVKEIPITHHVKE GYEKADPAQFELLKVLGQGSFGKVFLVRKKTGPDAGQLYAMKVLKKASL KVRDRVRTKMERDILVEVNHPFIVKLHYAFQTEGKLYLILDFLRGGDVF TRLSKEVLFTEEDVKFYLAELALALDHLHQLGIVYRDLKPENILLDEIG HIKLTDFGLSKESVDQEKKAYSFCGTVEYMAPEVVNRRGHSQSADWWSY GVLMEMLTGTLPFQGKDRNETMNMILKAKLGMPQFLSAEAQSLLRMLF KRN PANRLGSEGVEEIKRHLFFANIDWDKLYKREVQPPFKPASGKPPDDT FCFDPEFTAKTPKDSPGLPASANAHQLFKGFSFVATSIAEYKITPITS ANVLP IVQINGNAAQFGEVYELKEDIGVGSYSVCKRCIHATTNMEFAVK IIDKSKRDPSEEIEILMRYGQHPNIITLKDVFDDGRYVYLVTDLMKGGE LLDRILKQKCF SEREASDILYVISKTVDYLHCQGVVHRDLKPSN ILYMD ESASADSI R ICDFGFAKQLRGENGLLLTPCYTANFVAPEVLMQOQYDAA CDIWSLGVLFYTMLAGYTPFANGPNDTPEEILLRIGNGKFSLSGGNWDN ISDGAKDLLSHMLHMDPHQRYTAEQILKHSWITHRDQLPNDQPKRNDVS HVVKGAMVATYSAL THKTFQPVLEPVAASSLAQRSMKKRTSTGL</p>
<u>Native sequence</u>	<p>Amino acids M1 – L745 (end) of human RSK4. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<u>Protease cleavage</u>	PreScission (LEVL FQGP) residues 221 – 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pGEX6P-1

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Nucleotide Sequence Of Insert

ggatccATGCTACCATTCGCTCCTCAGGACGAGCCCTGGGACCGAGAAATGGAA
GTGTTcAGCGGGCGGCGGCGCAGCAGCGGCGAGGTAAATGGTCTTAAAATGGTT
GATGAGCCAATGGAAGAGGGAGAAGCAGATTCTTGTCATGATGAAGGAGTTGTT
AAAGAAATCCCTATTACTCATCATGTTAAGGAAGGCTATGAGAAAGCAGATCCT
GCACAGTTTGAGTTGCTCAAGGTTCTTGGTCAGGGGTCATTTGGAAAGGTTTTT
CTTGTTAGAAAGAAGACCGGTCCTGATGCTGGGCAGCTCTATGCAATGAAGGTG
TTAAAAAAGCCTCTTTAAAAGTTcGAGACAGAGTTCGGACAAAGATGGAGAGG
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CAGACTGAAGGGAAAAGTACTTAATACTGGATTTTCTCAGGGGAGGAGATGTT
TTCACAAGATTATCCAAAGAGGTTCTGTTTACAGAGGAAGATGTGAAATTCTAC
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