Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active RSK1 [1 - 735]

Enzyme description:- RSK1 [1 - 735]

Clone number:- DU 687

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6) and HA

Purification method:- Ni^{2+}-NTA agarose

Expression level:- 3-5 mg/L

Calculated molecular mass:- 88, 284 daltons

Purity:- >85 %

Activation protocol:-

RSK1 (2 µM) is activated by incubation with 5 U/ml GST-p42MAPKinase [DU 650 or DU1844] and 1 U/ml GST-PDK1 [DU 954] in 50mM Tris-HCl pH 7.5, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM magnesium acetate, 0.1 mM ATP for 30 min at 30 °C. Following activation, the active RSK1 is repurified by Ni^{2+}-NTA agarose chromatography.

Enzyme storage buffer:-

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

Storage temperature:- -70 °C

Assay:- Standard filter binding assay

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 % 2−mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-
Long S6 [KEAKEKRQEQIAKRRRLSSLRASTSKSGGSQK]
Final concentration: 30 µM

Specific activity range:- 300 – 600 U/mg

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Clone Data Sheet

RSK1 [1 - 735]

Protein RSK1 [1 - 735]

Clone number DU 687

Species Rat

Accession number M99169

Tags N-terminal His(6) and HA (YPYDVPDYA)

Baculovirus expressed protein

MSYYHHHHHHDYDIPTTENLYFQGAMDPEFAAATMYPYDVPDYALE**MPL**
AQLKEPWPLMELVPLDPENGQASGEEAGLQPSKDEGILKEISITHHVKA
GSEKADPSHFELLKVLGQGSFGKVFLVRKVTRPDNGHLYAMKVLKKATL
KVRDRVRTKMERDILADVNHPFVVKLHYAFQTEGKLYLILDFLRGGDLF
TRLSKEVMFTEEDVKFYLAELALGLDHLHSLGIIYRDLKPENILLDEEG
HIKLTDFGLSKEAIDHEKKAYSFCGTVEYMAPEVVNRQGHTHSADWWSY
GVLMFEMLTGSLPFQGKDRKETMTLILKAKLGMPQFLSTEAQSLLRALF
KRNPANRLGSGPDGAEEIKRHIFYSTIDWNKLYRREIKPPFKPAVAQPD
DTFYFDTEFTSRTPRDSPGIPPSAGAHQLFRGFSFVATGLMEDDSKPRA
TQAPLHSVVQQLHGKNLVFSDGYIVKETIGVGSYSVCKRCVHKATNMEY
AVKVIDKSKRDPSEEIEILLRYGQHPNIITLKDVYDDSKHVYLVTELMR
GGELLDKILRQKFFSEREASFVLYTISKTVEYLHSQGVVHRDLKPSNIL
YVDESGNPG**CLRICDFGFAKQLRAENGLLMTPCYTANFVAPEVLKRQGY**
DEGCDIWSLGVLLYTMLAGYTPFANGPSDTPEEILTRISSGKFTLN**GGN**
WNTVSETAKDLVSKMLHVDPHQRLTAKQVLQHPWITQKDKLPQSQLSHQ
DLQLVKGA**MAATYSALSSSKPTPQLKPIESSILAQRRVRKLPSTTL**

Native sequence

Amino acids M1 – L735 (end) of rat RSK1.
Residue M47 of the fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 5 – 10 and the HA tag is located at residues 36 – 44.

The following amino acid ubstitutions are present:
E – G, where E551 of the native sequence is G597 of the fusion protein.
S – N, where S637 of the native sequence is N683 of the fusion protein.
G – A, where G697 of the native sequence is A743 of the fusion protein.
The additional glutamine, Q157 (native sequence) reported in Dalby *et al.* (1998) *J.Biol.Chem.* **273**, 1496-1998, has been removed from the fusion protein.

Protease cleavage rTEV (ENLYFQG) residues 18 - 24

Cloning sites *Not*1 site in pFastBAC HTb

Complete nucleotide sequence

ATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCAACGA
CCGAAAACCTGTATTTTCAGGGCGCCATGGATCCGGAATTCGCGGCCGC
CACCATGTACCCATACGATGTTCCAGATTACGCTCTCGAGATGCCGCTC
GCCCAGCTCAAGGAACCCTGGCCGCTCATGGAGCTGGTGCCGCTGGACC
CGGAGAATGGACAGGCTTCAGGGGAAGAAGCTGGACTTCAGCCATCCAA
GGATGAGGGCATCCTCAAGGAGATCTCTATCACACACCACGTCAAGGCT
GGCTCTGAGAAGGCTGATCCATCCCATTTTGAGCTCCTCAAGGTTCTGG
GCCAAGGATCCTTTGGCAAAGTCTTCCTGGTACGCAAGGTCACCCGGCC
TGACAATGGGCACTTGTATGCCATGAAAGTATTAAAGAAGGCCACGCTG
AAAGTGCGTGACCGTGTTCGGACCAAGATGGAGAGAGACATCCTAGCTG
ACGTGAACCACCCCTTCGTAGTGAAACTGCACTATGCCTTCCAGACCGA
GGGCAAGCTCTATCTTATTCTGGACTTTCTGCGTGGTGGAGACCTGTTC
ACACGACTCTCAAAGGAGGTTATGTTTACAGAGGAGGATGTGAAGTTTT
ACCTGGCTGAGCTGGCACTGGGCCTGGACCACCTGCACAGCTTGGGCAT
CATTTACAGAGACCTCAAGCCTGAAAATATCCTTTTGGATGAGGAGGGC
CACATCAAACTCACTGACTTTGGCCTGAGCAAGGAGGCCATTGACCACG
AAAAGAAGGCCTATTCCTTCTGCGGGACGGTGGAGTACATGGCCCCCGA
GGTTGTCAACCGCCAGGGCCACACCCACAGTGCAGATTGGTGGTCCTAT
GGGGTGTTGATGTTTGAGATGCTGACGGGCTCCCTGCCCTTCCAGGGGA
AGGACCGGAAGGAGACCATGACCTTGATTTTGAAGGCAAAGCTAGGCAT
GCCCCAGTTTCTGAGCACGGAAGCCCAGAGCCTCCTGCGGGCCCTGTTC
AAGAGGAATCCTGCCAACCGGCTTGGCTCAGGCCCCGATGGGGCTGAGG
AAATTAAGAGACATATCTTCTACTCTACCATTGACTGGAATAAGCTCTA
CCGCCGTGAGATCAAGCCACCTTTCAAGCCCGCTGTGGCCCAACCGGAT
GACACCTTCTACTTTGATACCGAGTTCACGTCACGCACACCCAGGGATT
CGCCGGGCATCCCCCCCAGTGCTGGTGCCCATCAGCTCTTCCGTGGCTT
CAGCTTCGTGGCCACCGGTCTGATGGAGGATGACAGCAAGCCTCGGGCC
ACCCAAGCTCCGCTGCACTCGGTGGTACAGCAACTCCACGGGAAGAACT
TGGTTTTCAGCGATGGCTACATAGTAAAGGAGACGATCGGCGTGGGCTC
CTACTCTGTGTGTAAGCGCTGTGTCCACAAGGCCACCAACATGGAGTAC
GCAGTCAAAGTCATCGACAAAAGCAAAAGAGATCCCTCCGAAGAGATCG
AGATTCTTCTGCGGTATGGACAGCACCCCAACATCATCACCCTGAAAGA
TGTGTATGACGACAGTAAGCACGTATACCTGGTGACAGAGTTGATGAGG
GGCGGGGAGCTGCTGGATAAGATCCTACGGCAGAAATTCTTCTCAGAGC
GGGAGGCCAGCTTCGTCCTGTACACCATCAGCAAGACTGTGGAATACTT
GCACTCCCAAGGGGTCGTCCACAGGGACCTCAAACCCAGTAACATCCTG
TATGTGGATGAGTCTGGGAACCCCGGATGCCTACGAATATGCGACTTTG
GCTTTGCCAAGCAGCTACGGGCTGAGAACGGGCTTCTCATGACACCTTG
CTACACAGCCAACTTTGTGGCACCTGAGGTGCTGAAGCGTCAGGGCTAC
GATGAAGGCTGTGACATATGGAGCCTGGGCGTTCTGCTGTACACGATGC
TGGCAGGATACACTCCATTTGCCAATGGGCCCAGTGATACCCCAGAGGA
GATCCTCACCCGGATCAGCAGTGGGAAGTTCACCCTCAACGGGGGAAAC
TGGAACACGGTTTCAGAGACAGCCAAGGACTTAGTATCTAAGATGCTGC
ATGTGGACCCCCACCAGCGCCTCACAGCCAAACAGGTTCTGCAGCACCC
GTGGATCACCCAGAAAGACAAGCTCCCCCAGAGCCAGTTGTCCCACCAA
GACCTGCAGCTTGTGAAGGGGGCCATGGCAGCTACATATTCTGCACTCA
GTAGCTCCAAACCCACCCCCCAGCTCAAGCCAATCGAGTCGTCCATCCT
GGCCCAGCGGCGGGTGAGGAAGCTGCCATCCACCACCCTG

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