Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active RSK1 [1 - 735]

Enzyme description:- RSK1 [1 - 735]

Clone number:- DU 19046

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

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

Calculated molecular mass:-
Monoisotopic 86, 384.33 daltons
Average Mass 86, 4438.98 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.80

Purity:- >85 %

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 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

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Clone Data Sheet

RSK1 [1 - 735]

Protein	RSK1 [1 - 735]
Clone number	DU 19046
Species	Human
Accession number	NM_002953.3
Tags	N-terminal His(6)
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMDPEF**MPLAQLKEPWPLMELVPLD PENGQTSGEEAGLQPSKDEGVLKEISITHHVKAGSEKADPSHFELLKVL GQGSFGKVFLVRKVTRPDSGHLYAMKVLKKATLKVRDRVRTKMERDILA DVNHPFVVKLHYAFQTEGKLYLILDFLRGGDLFTRLSKEVMFTEEDVKF YLAELALGLDHLHSLGIIYRDLKPENILLDEEGHIKLTDFGLSKEAIDH EKKAYSFCGTVEYMAPEVVNRQGHSHSADWWSYGVLMFEMLTGSLPFQG KDRKETMTLILKAKLGMPQFLSTEAQSLLRALFKRNPANRLGSGPDGAE EIKRHVFYSTIDWNKLYRREIKPPFKPAVAQPDDTFYFDTEFTSRTPKD SPGIPPSAGAHQLFRGFSFVATGLMEDDGKPRAPQAPLHSVVQQLHGKN LVFSDGYVVKETIGVGSYSECKRCVHKATNMEYAVKVIDKSKRDPSEEI EILLRYGQHPNIITLKDVYDDGKHVYLVTELMRGGELLDKILRQKFFSE REASFVLHTIGKTVEYLHSQGVVHRDLKPSNILYVDESGNPECLRICDF GFAKQLRAENGLLMTPCYTANFVAPEVLKRQGYDEGCDIWSLGILLYTM LAGYTPFANGPSDTPEEILTRIGSGKFTLSGGNWNTVSETAKDLVSKML HVDPHQRLTAKQVLQHPWVTQKDKLPQSQLSHQDLQLVKGAMAATYSAL NSSKPTPQLKPIESSILAQRRVRKLPSTTL**
Native sequence	Amino acids M1 – L735 (end) of human RSK1. Residue M32 of the fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 5 – 10.
Protease cleavage	rTEV (ENLYFQG) residues 18 - 24
Cloning sites	*Eco*R1 and *Not*1 site in pFastBAC HTa

Nucleotide sequence of insert

gaattcATGCCGCTCGCCCAGCTCAAGGAGCCCTGGCCGCTCATGGAGC
TAGTGCCTCTGGACCCGGAGAATGGACAGACCTCAGGGGAAGAAGCTGG
ACTTCAGCCGTCCAAGGATGAGGGCGTCCTCAAGGAGATCTCCATCACG
CACCACGTCAAGGCTGGCTCTGAGAAGGCTGATCCATCCCATTTCGAGC
TCCTCAAGGTTCTGGGCCAGGGATCCTTTGGCAAAGTCTTCCTGGTGCG
GAAAGTCACCCGGCCTGACAGTGGGCACCTGTATGCTATGAAGGTGCTG
AAGAAGGCAACGCTGAAAGTACGTGACCGCGTCCGGACCAAGATGGAGA
GAGACATCCTGGCTGATGTAAATCACCCATTCGTGGTGAAGCTGCACTA
TGCCTTCCAGACCGAGGGCAAGCTCTATCTCATTCTGGACTTCCTGCGT
GGTGGGGACCTCTTCACCCGGCTCTCAAAAGAGGTGATGTTCACGGAGG
AGGATGTGAAGTTTTACCTGGCCGAGCTGGCTCTGGGCCTGGATCACCT
GCACAGCCTGGGTATCATTTACAGAGACCTCAAGCCTGAGAACATCCTT
CTGGATGAGGAGGGCCACATCAAACTCACTGACTTTGGCCTGAGCAAAG
AGGCCATTGACCACGAGAAGAAGGCCTATTCTTTCTGCGGGACAGTGGA
GTACATGGCCCCTGAGGTCGTCAACCGCCAGGGCCACTCCCATAGTGCG
GACTGGTGGTCCTATGGGGTGTTGATGTTTGAGATGCTGACGGGCTCCC
TGCCCTTCCAGGGGAAGGACCGGAAGGAGACCATGACACTGATTCTGAA
GGCGAAGCTAGGCATGCCCCAGTTTCTGAGCACTGAAGCCCAGAGCCTC
TTGCGGGCCCTGTTCAAGCGGAATCCTGCCAACCGGCTCGGCTCCGGCC
CTGATGGGGCAGAGGAAATCAAGCGGCATGTCTTCTACTCCACCATTGA
CTGGAATAAGCTATACCGTCGTGAGATCAAGCCACCCTTCAAGCCAGCA
GTGGCTCAGCCTGATGACACCTTCTACTTTGACACCGAGTTCACGTCCC
GCACACCCAAGGATTCCCCAGGCATCCCCCCCAGCGCTGGGGCCCATCA
GCTGTTCCGGGGCTTCAGCTTCGTGGCCACCGGCCTGATGGAAGACGAC
GGCAAGCCTCGTGCCCCGCAGGCACCCCTGCACTCGGTGGTACAGCAAC
TCCATGGGAAGAACCTGGTTTTTAGTGACGGCTACGTGGTAAAGGAGAC
AATTGGTGTGGGCTCCTACTCTGAGTGCAAGCGCTGTGTCCACAAGGCC
ACCAACATGGAGTATGCTGTCAAGGTCATTGATAAGAGCAAGCGGGATC
CTTCAGAAGAGATTGAGATTCTTCTGCGGTATGGCCAGCACCCCAACAT
CATCACTCTGAAAGATGTGTATGATGATGGCAAACACGTGTACCTGGTG
ACAGAGCTGATGCGGGGTGGGGAGCTGCTGGACAAGATCCTGCGGCAGA
AGTTCTTCTCAGAGCGGGAGGCCAGCTTTGTCCTGCACACCATTGGCAA
AACTGTGGAGTATCTGCACTCACAGGGGGTTGTGCACAGGGACCTGAAG
CCCAGCAACATCCTGTATGTGGACGAGTCCGGGAATCCCGAGTGCCTGC
GCATCTGTGACTTTGGTTTTGCCAAACAGCTGCGGGCTGAGAATGGGCT
CCTCATGACACCTTGCTACACAGCCAACTTTGTGGCGCCTGAGGTGCTG
AAGCGCCAGGGCTACGATGAAGGCTGCGACATCTGGAGCCTGGGCATTC
TGCTGTACACCATGCTGGCAGGATATACTCCATTTGCCAACGGTCCCAG
TGACACACCAGAGGAAATCCTAACCCGGATCGGCAGTGGGAAGTTTACC
CTCAGTGGGGGAAATTGGAACACAGTTTCAGAGACAGCCAAGGACCTGG
TGTCCAAGATGCTACACGTGGATCCCCACCAGCGCCTCACAGCTAAGCA
GGTTCTGCAGCATCCATGGGTCACCCAGAAAGACAAGCTTCCCCAAAGC
CAGCTGTCCCACCAGGACCTACAGCTTGTGAAGGGAGCCATGGCTGCCA
CGTACTCCGCACTCAACAGCTCCAAGCCCACCCCCCAGCTGAAGCCCAT
CGAGTCATCCATCCTGGCCCAGCGGCGAGTGAGGAAGTTGCCATCCACC
ACCCTGtgagcggccgc

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