Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active RSK3 [2 - 733]

Enzyme description:- RSK3 [2 - 733]

Clone number:- DU 902

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 1.5 mg/L

Calculated molecular mass:- 110, 248 daltons

Purity:- 70 %

Activation protocol:-

RSK3 (2 µM) is activated by incubation with 5 U/ml GST-p42MAPKinase [DU 650 or DU 1844] and 3.3 µg/ml GST-PDK1 [DU 954] in 50 mM 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.

At present we are unable to remove the upstream activators from the RSK3.

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, 0.2 mM PMSF, 1 mM Benzamidine

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:- to be determined

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Clone Data Sheet

RSK3 [2 - 733]

Protein	RSK3 [2 - 733]
Clone number	DU 902
Species	Human
Accession number	X85106
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEF**DLSMKK FAVRRFFSVYLRRKSRSKSSSLSRLEEEGVVKEIDISHHVKEGFEKAD PSQFELLKVLGQGSYGKVFLVRKVKGSDAGQLYAMKVLKKATLKVRDR VRSKMERDILAEVNHPFIVKLHYAFQTEGKLYLILDFLRGGDLFTRLS KEVMFTEEDVKFYLAELALALDHLHSLGIIYRDLKPENILLDEEGHIK ITDFGLSKEAIDHDKRAYSFCGTIEYMAPEVVNRRGHTQSADWWSFGV LMFEMLTGSLPFQGKDRKETMALILKAKLGMPQFLSGEAQSLLRALFK RNPCNRLGAGIDGVEEIKRHPFFVTIDWNTLYRKEIKPPFKPALGRPE DTFHFDPEFTARTPTDSPGVPPSANAHHLFRGFSFVASSLIQEPSQQD LHKVPVHPIVQQLHGNNIHFTDGYEIKEDIGVGSYSVCKRCVHKATDT EYAVKIIDKSKRDPSEEIEILLRYGQHPNIITLKDVYDDGKFVYLVME LMRGGELLDRILRQRYFSEREASDVLCTITKTMDYLHSQGVVHRDLKP SNILYRDESGSPESIRVCDFGFAKQLRAGNGLLMTPCYTANFVAPEVL KRQGYDAACDIWSLGILLYTMLAGFTPFANGPDDTPEEILARIGSGKY ALSGGNWDSISDAAKDVVSKMLHVDPHQRLTAMQVLKHPWVVNREYLS PNQLSRQDVHLVKGAMAATYFALNRTPQAPRLEPVLSSNLAQRRGMKR LTSTRL**
Native sequence	Amino acids D2 – L733 (end) of human RSK3. Residue D235 of the fusion protein is equilivalent to D2 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGPL) at residues 221 – 229
Cloning sites	*Eco*R1 and *Sal*1 site of pGEX6P-1

Nucleotide sequence of insert

ATGGACCTGAGCATGAAGAAGTTCGCCGTGCGCAGGTTCTTCTCTGTG
TACCTGCGCAGGAAGTCGCGCTCCAAGAGCTCCAGCCTGAGCCGGCTC
GAGGAAGAAGGTGTCGTGAAGGAGATAGACATCAGCCATCATGTGAAG
GAGGGCTTTGAGAAGGCAGATCCTTCCCAGTTTGAGCTGCTGAAGGTT
TTAGGACAAGGATCCTATGGAAAGGTGTTCCTGGTGAGGAAGGTGAAG
GGGTCCGACGCTGGGCAGCTCTACGCCATGAAGGTCCTTAAGAAAGCC
ACCCTAAAAGTTCGGGACCGAGTGAGATCGAAGATGGAGAGAGACATC
TTGGCAGAAGTGAATCACCCCTTCATTGTGAAGCTTCATTATGCCTTT
CAGACGGAAGGAAAGCTCTACCTGATCCTGGACTTCCTGCGGGGAGGG
GACCTCTTCACCCGGCTCTCCAAAGAGGTCATGTTCACGGAGGAGGAT
GTCAAGTTCTACCTGGCTGAGCTGGCCTTGGCTTTAGACCATCTCCAC
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GATGAAGAGGGGCACATTAAGATCACAGATTTCGGCCTGAGTAAGGAG
GCCATTGACCACGACAAGAGAGCGTACTCCTTCTGCGGGACGATCGAG
TACATGGCACCCGAGGTGGTGAACCGGCGAGGACACACGCAGAGTGCC
GACTGGTGGTCCTTCGGCGTGCTCATGTTTGAGATGCTCACGGGGTCC
CTGCCGTTCCAGGGGAAGGACAGGAAGGAGACCATGGCTCTCATCCTC
AAAGCCAAGCTGGGGATGCCGCAGTTCCTCAGTGGGGAGGCACAGAGT
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ACCCCTTTTGCAAATGGGCCAGACGATACCCCTGAGGAGATTCTGGCG
CGGATCGGCAGTGGGAAGTATGCCCTTTCTGGGGGAAACTGGGACTCG
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CCTCATCAGCGCCTGACGGCGATGCAAGTGCTCAAACACCCGTGGGTG
GTCAACAGAGAGTACCTGTCCCCAAACCAGCTCAGCCGACAGGACGTG
CACCTGGTGAAGGGCGCGATGGCCGCCACCTACTTTGCTCTAAACAGA
ACACCTCAGGCCCCGCGGCTGGAGCCCGTGCTGTCATCCAACCTGGCT
CAGCGCAGAGGCATGAAGAGACTCACGTCCACGCGGCTGtag