University of Dundee

Standard Operating Procedure

Preparation of active RSK2 [2 - 740]

Enzyme description:- RSK2 [2 - 740]

Clone number:- DU 1846

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

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

Expression level:- 5-10 mg/L

Calculated molecular mass:- 84, 678 daltons

Purity:- >95 %

Activation protocol:-

RSK2 (2 μM) is activated by incubation with 5 U/ml GST-p42MAPKinase [DU 650 or DU 1844] and 1 U/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. Following activation, the active MAPKAP-K1b 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:- 500 – 1000 U/mg

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Clone Data Sheet -RSK2 [2 - 740]

Protein	RSK2 [2 - 740]
Clone number	DU 1846
Species	Human
Accession number	NM_004586
Tags	N-terminal His(6)
Baculovirus expressed protein	MAHHHHHHGS**PLAQLADPWQKMAVESPSDSAENGQQIMDEPMGEEEINP QTEEGSIKEIAITHHVKEGHEKADPSQFELLKVLGQGSFGKVFLVKKIS GSDARQLYAMKVLKKATLKVRDRVRTKMERDILVEVNHPFIVKLHYAFQ TEGKLYLILDFLRGGDLFTRLSKEVMFTEEDVKFYLAELALALDHLHSL GIIYRDLKPENILLDEEGHIKLTDFGLSKESIDHEKKAYSFCGTVEYMA PEVVNRRGHTQSADWWSFGVLMFEMLTGTLPFQGKDRKETMTMILKAKL GMPQFLSPEAQSLLRMLFKRNPANRLGAGPDGVEEIKRHSFFSTIDWNK LYRREIHPPFKPATGRPEDTFYFDPEFTAKTPKDSPGIPPSANAHQLFR GFSFVAITSDDESQAMQTVGVHSIVQQLHRNSIQFTDGYEVKEDIGVGS YSVCKRCIHKATNMEFAVKIIDKSKRDPTEEIEILLRYGQHPNIITLKD VYDDGKYVYVVTELMKGGELLDKILRQKFFSEREASAVLFTITKTVEYL HAQGVVHRDLKPSNILYVDESGNPESIRICDFGFAKQLRAENGLLMTPC YTANFVAPEVLKRQGYDAACDIWSLGVLLYTMLTGYTPFANGPDDTPEE ILARIGSGKFSLSGGYWNSVSDTAKDLVSKMLHVDPHQRLTAALVLRHP WIVHWDQLPQYQLNRQDAPHLVKGAMAATYSALNRNQSPVLEPVGRSTL AQRRGIKKITSTAL**
Native sequence	Amino acids P2 – L740 (end) of human RSK2. Residue P11 of the fusion protein is equilivalent to P2 of the native enzyme. The His(6) tag is located at residues 3 – 8. The following amino acid substitution is present: V – G, where V45 of the native sequence is G54 of the fusion protein.
Protease cleavage	None
Cloning sites	*Nde*1 and *Xho*1 sites of modified pFastBAC 1.

Complete nucleotide sequence

ATGGCGCATCACCATCACCATCACGGATCCCCGCTGGCGCAGCTGGCGG
ACCCGTGGCAGAAGATGGCTGTGGAGAGCCCTTCCGACAGCGCGGAGAA
TGGACAGCAAATTATGGATGAACCTATGGGAGAGGAGGAGATTAACCCA
CAAACTGAAGAAGGCAGTATCAAAGAAATTGCAATCACACATCATGTGA
AGGAAGGACATGAAAAGGCAGATCCTTCCCAGTTTGAACTTTTAAAAGT
ATTAGGGCAGGGATCATTTGGAAAGGTTTTCTTAGTTAAAAAAATCTCA
GGCTCTGATGCTAGACAGCTTTATGCCATGAAAGTATTAAAGAAGGCCA
CGCTGAAAGTTCGAGACCGTGTTCGGACAAAAATGGAACGTGATATCTT
GGTAGAAGTCAATCACCCTTTCATTGTCAAATTGCATTACGCTTTTCAA
ACGGAAGGGAAGTTGTATCTTATTTTGGATTTTCTCAGGGGCGGAGACT
TGTTTACACGCTTATCCAAAGAGGTGATGTTCACAGAGGAAGATGTCAA
ATTCTACTTGGCTGAACTTGCACTTGCTTTAGACCATCTTCATAGCCTG
GGAATAATCTATAGAGACTTAAAACCAGAAAACATACTTCTTGATGAAG
AAGGTCACATCAAGTTAACTGATTTTGGCTTAAGTAAGGAATCTATTGA
TCATGAGAAGAAGGCTTATTCTTTTTGTGGCACTGTGGAATACATGGCT
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GGGATGCCACAGTTTCTGAGTCCTGAAGCCCAGAGTCTTTTACGAATGC
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TGAAGAAATTAAAAGACATTCATTTTTCTCAACAATAGACTGGAATAAA
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AGAGGCCAGCGCTGTCCTGTTTACTATAACCAAAACTGTTGAGTATCTT
CATGCACAAGGGGTGGTTCACAGAGACTTGAAACCTAGCAACATTCTTT
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ATGCGCCGCATCTCGTAAAGGGTGCCATGGCAGCTACGTACTCTGCTTT
AAACCGCAATCAGTCCCCAGTCTTGGAACCAGTGGGCCGCTCCACTCTT
GCTCAGCGGAGAGGGATTAAAAAAATCACCTCAACAGCCCTGtga