Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active ROCK II [11 – 552]

Enzyme description:- ROCK II [11 – 552]

Clone number:- DU 19085

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6) tag

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

Calculated molecular mass:-
Monoisotopic 65, 612.53 daltons
Average Mass 65, 654.10 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.35

Purity:- 80 %

Activation protocol:- Constitutively active

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

Substrate:-
Long S6 peptide (KEAKEKRQEQIAKRRRLSSLRASTSKSGGSQK)
Final concentration: 30 μM

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Clone Data Sheet

ROCK II [11 – 552]

Protein	ROCK II [11 - 552]
Clone number	DU 19085
Species	Human
Accession number	NM_004850
Tags	N-terminal His(6)
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGS**PGAPETAPGDGAGASRQ** **RKLEALIRDPRSPINVESLLDGLNSLVLDLDFPALRKNKNIDNFL** **NRYEKIVKKIRGLQMKAEDYDVVKVIGRGAFGEVQLVRHKASQKV** **YAMKLLSKFEMIKRSDSAFFWEERDIMAFANSPWVVQLFYAFQDD** **RYLYMVMEYMPGGDLVNLMSNYDVPEKWAKFYTAEVVLALDAIHS** **MGLIHRDVKPDNMLLDKHGHLKLADFGTCMKMDETGMVHCDTAVG** **TPDYISPEVLKSQGGDGFYGRECDWWSVGVFLYEMLVGDTPFYAD** **SLVGTYSKIMDHKNSLCFPEDAEISKHAKNLICAFLTDREVRLGR** **NGVEEIRQHPFFKNDQWHWDNIRETAAPVVPELSSDIDSSNFDDI** **EDDKGDVETFPIPKAFVGNQLPFIGFTYYRENLLLSDSPSCRETD** **SIQSRKNEESQEIQKKLYTLEEHLSNEMQAKEELEQKCKSVNTRL** **EKTAKELEEEITLRKSVESALRQLEREKALLQHKNAEYQRKADHE** **ADKKRNLENDVNSLKDQLEDLKKRNQNSQI**
Native sequence	Amino acids P11 – I552 of human ROCKII. [Full length protein ends at residue S1388] Residue P29 of the fusion protein is equivalent to P11 of the native enzyme. The His(6) tag is located at residues 5 - 10.
Protease cleavage	rTEV (ENLYFQG) residues 18 - 24
Cloning sites	*Bam*H1 and *Eco*R1 sites of pFB-HTb

Nucleotide Sequence of Insert

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ggatccCCCGGCGCCCCCGAGACCGCGCCGGGGGACGGGGCAGGC
GCGAGCCGCCAGAGGAAGCTGGAGGCGCTGATCCGAGACCCTCGC
TCCCCCATCAACGTGGAGAGCTTGCTGGATGGCTTAAATTCCTTG
GTCCTTGATTTAGATTTTCCTGCTTTGAGGAAAAACAAGAACATA
GATAATTTCTTAAATAGATATGAGAAAATTGTGAAAAAAATCAGA
GGTCTACAGATGAAGGCAGAAGACTATGATGTTGTAAAAGTTATT
GGAAGAGGTGCTTTTGGTGAAGTGCAGTTGGTTCGTCACAAGGCA
TCGCAGAAGGTTTATGCTATGAAGCTTCTTAGTAAGTTTGAAATG
ATAAAAAGATCAGATTCTGCCTTTTTTTGGGAAGAAAGAGATATT
ATGGCCTTTGCCAATAGCCCCTGGGTGGTTCAGCTTTTTTATGCC
TTTCAAGATGATAGGTATCTGTACATGGTAATGGAGTACATGCCT
GGTGGAGACCTTGTAAACCTTATGAGTAATTATGATGTGCCTGAA
AAATGGGCCAAATTTTACACTGCTGAAGTTGTTCTTGCTCTGGAT
GCAATACACTCCATGGGTTTAATACACAGAGATGTGAAGCCTGAC
AACATGCTCTTGGATAAACATGGACATCTAAAATTAGCAGATTTT
GGCACGTGTATGAAGATGGATGAAACAGGCATGGTACATTGTGAT
ACAGCAGTTGGAACACCGGATTATATATCACCTGAGGTTCTGAAA
TCACAAGGGGGTGATGGTTTCTATGGGCGAGAATGTGATTGGTGG
TCTGTAGGTGTTTTCCTTTATGAGATGCTAGTGGGGGATACTCCA
TTTTATGCGGATTCACTTGTAGGAACATATAGCAAAATTATGGAT
CATAAGAATTCACTGTGTTTCCCTGAAGATGCAGAAATTTCCAAA
CATGCAAAGAATCTCATCTGTGCTTTCTTAACAGATAGGGAGGTA
CGACTTGGGAGAAATGGGGTGGAAGAAATCAGACAGCATCCTTTC
TTTAAGAATGATCAGTGGCATTGGGATAACATAAGAGAAACGGCA
GCTCCTGTAGTACCTGAACTCAGCAGTGACATAGACAGCAGCAAT
TTCGATGACATTGAAGATGACAAAGGAGATGTAGAAACCTTCCCA
ATTCCTAAAGCTTTTGTTGGAAATCAGCTGCCTTTCATCGGATTT
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ACCTTACGGAAAAGTGTGGAATCAGCATTAAGACAGTTAGAAAGA
GAAAAGGCGCTTCTTCAGCACAAAAATGCAGAATATCAGAGGAAA
GCTGATCATGAAGCAGACAAAAAACGAAATTTGGAAAATGATGTT
AACAGCTTAAAAGATCAACTTGAAGATTTGAAAAAAAGAAATCAA
AACTCTCAAATAtgacggccgcctagaagggcgaattc
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