

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of active ROCK1 [1 - 535]**

<b><u>Enzyme description:-</u></b>	ROCK1 [1 – 535]
<b><u>Clone number:-</u></b>	DU 51657
<b><u>Source:-</u></b>	Recombinant
<b><u>Expression system:-</u></b>	Baculovirus expression vector system
<b><u>Tag:-</u></b>	N-terminal GST
<b><u>Purification method:-</u></b>	GSH Sepharose

#### **Calculated molecular mass:-**

Monoisotopic        89, 981.87 daltons  
Average Mass        90, 039.56 daltons  
[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-**                    5.29

**Purity:-**                                >80 %

**Activation protocol:-**                Constitutively active

#### **Enzyme storage buffer:-**

50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM 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 mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc

#### **Substrate:-**

Long S6 peptide (KEAKEKRQEQIAKRRRLSSLRASTSKSGGSQK)

Final concentration: 30 µM

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**Clone Data Sheet**

**ROCK1 [1 - 535]**

**Protein** ROCK1 [1 - 535]

**Clone number** DU 51657

**Species** Human

**Accession number** NM\_005406.2

**Tags** N-terminal GST

**Baculovirus  
expressed protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFEL  
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLE  
GAVLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLN  
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY  
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFGPLGSPGIPGSTR  
AAMSTGDSFETRFEKMDNLLRDPKSEVNSDCLLDGLDALVYDLDFPAL  
RKNKNIDNFLSRYKDTINKIRDLRMKAEDYEVVKVIGRGAFGEVQLVR  
HKSTRKVYAMKLLSKFEMIKRSDSAFFWEERDIMAFANSPWVQLFYA  
FQDDRYLYMVEYMPGGDLVNLMSNYDVPEKWARFYTAEVVLALDAIH  
SMGFIHRDVKPDNMLLDKSGHLKLADFGTCMKMKEGMVRCDTAVGTP  
DYISPEVLKSQGGDGYYGRECDWWSVGVFLYEMLVGDTPFYADSLVGT  
YSKIMNHKNSLTFPDDNDISKEAKNLICAFLLTDREVR LGRNGVEEIKR  
HLFFKNDQWAWETLRDTVAPVVPDLSSDIDTSNFDDLEEDKGEETFP  
IPKAFVGNQLPFVGFYYSNRRYLSSANPNDNRTSSNADKSLQESLQK  
TIYKLEEQLHNEMQLKDEMEQKCRTSNIKLDKIMKELDEEGNQRRNLE  
STVSQIEKEKMLLQHRINEYQRKAEQENEKRRNVENEVSTLKDQLEDL  
KKVSQNSQL

**Native sequence** Amino acids M1 – L535 (end residue S1354) of human ROCK1.  
Residue M243 of the fusion protein is equivalent to M1 of the native  
enzyme. The GST tag is located at residues 1 – 220.

**Protease cleavage** PreScission site (LEVLFQGP) residues 221 – 228

**Cloning sites** *Not1* sites of pFastBac GST 6P2

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Nucleotide  
sequence of  
insert

gcggccgcgATGTCGACTGGGGACAGTTTTGAGACTCGATTTGAAAA  
ATGGACAACCTGCTGCGGGATCCCAAATCGGAAGTGAATTCGGATTGT  
TTGCTGGATGGATTGGATGCTTTGGTATATGATTTGGATTTTCCTGCC  
TTAAGAAAAACAAAAATATTGACAACTTTTTAAGCAGATATAAAGAC  
ACAATAAAATAAAATCAGAGATTTACGAATGAAAGCTGAAGATTATGAA  
GTAGTGAAGGTGATTGGTAGAGGTGCATTTGGAGAAGTTCAATTGGTA  
AGGCATAAATCCACCAGGAAGGTATATGCTATGAAGCTTCTCAGCAAA  
TTTGAAATGATAAAGAGATCTGATTCTGCTTTTTTCTGGGAAGAAAGG  
GACATCATGGCTTTTGCCAACAGTCCTTGGGTTGTTTCAGCTTTTTTAT  
GCATTCCAAGATGATCGTTATCTCTACATGGTGTGGAATACATGCCT  
GGTGGAGATCTTGTAACCTTAATGAGCAACTATGATGTGCCTGAAAA  
TGGGCACGATTCTATACTGCAGAAGTAGTTCCTGCATTGGATGCAATC  
CATCCATGGGTTTTATTACAGAGATGTGAAGCCTGATAACATGCTG  
CTGGATAAATCTGGACATTTGAAGTTAGCAGATTTTGGTACTTGTATG  
AAGATGAATAAGGAAGGCATGGTACGATGTGATACAGCGGTTGGAACA  
CCTGATTATATTTCCCTGAAGTATTAATAATCCCAAGGTGGTGTGGT  
TATTATGGAAGAGAATGTGACTGGTGGTCGGTTGGGGTATTTTTATAC  
GAAATGCTTGTAGGTGATACACCTTTTTTATGCAGATTCCTTGGTTGGA  
ACTTACAGTAAAAATTATGAACCATAAAAAATTCACCTTACCTTTCCTGAT  
GATAATGACATATCAAAAAGCAAAAAACCTTATTTGTGCCTTTCCTT  
ACTGACAGGGAAGTGAGGTTAGGGCGAAATGGTGTAGAAGAAATCAAA  
CGACATCTCTTCTTCAAAAATGACCAGTGGGCTTGGGAAACGCTCCGA  
GACACTGTAGCACCAGTTGTACCCGATTTAAGTAGTGACATTGATACT  
AGTAATTTTGATGACTTGGGAAGAAGATAAAGGAGAGGAAGAAACATTC  
CCTATTCCTAAAGCTTTTCGTTGGCAATCAACTACCTTTTGTAGGATTT  
ACATATTATAGCAATCGTAGATACTTATCTTCAGCAAATCCTAATGAT  
AACAGAACTAGCTCCAATGCAGATAAAAGCTTGCAGGAAAGTTTGCAA  
AAAACAATCTATAAGCTGGAAGAACAGCTGCATAATGAAATGCAGTTA  
AAAGATGAAATGGAGCAGAAGTGCAGAACCTCAAACATAAAACTAGAC  
AAGATAATGAAAGAATTGGATGAAGAGGGAAATCAAAGAAGAAATCTA  
GAATCTACAGTGTCTCAGATTGAGAAGGAGAAAATGTTGCTACAGCAT  
AGAATTAATGAGTACCAAAGAAAAGCTGAACAGGAAAATGAGAAGAGA  
AGAAATGTAGAAAATGAAGTTTCTACATTAAGGATCAGTTGGAAGAC  
TTAAAGAAAGTCAGTCAGAATTCACAGCTTtaagcggccgc