

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of srGAP1 [1 – 1085]

Enzyme description:- srGAP1 [1 – 1085]

Clone number:- DU 7137

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 150, 992.96 daltons

Average Mass 151, 088.46 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.25

Purity:- >80 %

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

srGAP1 [1 – 1085]

Protein srGAP1 [1 – 1085]

Clone number DU 7137

Species Human

Accession number BC053903.1

Tags N-terminal GST

Bacterially expressed protein MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAESMLEGA
VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFOGPLGSMSTPSRFKKDKKEII
AEYESQVKEIRAQLVEQQKCLEQQTEMRVQLLQDLQDFFRKKAEIET EY
SRNLEKLAERFMAKTRSTKDHQQYKKDQNLSPVNCWYLLLNQVRRESK
DHATLSDIYLNNVIMRFMQISEDSTRMFKKSKEIAFQLHEDLMKVLNEL
YTVMKTYHMYHAESISAESKLKEAEKQEEKQIGRSGDPVFHIRLEERHQ
RRSSVKKIEKMKEKRQAKYSENKLSIKARNEYLLTLEATNASVFKYYI
HDLSDLIDCCDLGYHASLNRLR TYLSAEYNLETSRHEGLDIIENAVDN
LEPRSDKQRFMEMYPAAF C PPMKFEFQSHMGDEVQVSAQQPVQAEML
RYQQLOSRLATLKIENEEVKKTTTEATLQTIQDMVTIEDYDVSECFQHSR
STESVKSTVSETYLSKPSIAKRANQOETE QFYFMKLREYLEGSNLITK
LQAKHDLLQRTLGE GHRAEYMTTRPPNVPKPKQKHKRSRPRSQYNTKLF
NGDLETFVKDSGQVIPLIVESCIRFINLYGLOHQGIFRVSGSQVEVNDI
KNSFERGENPLADDQSNHDINSVAGVLKLYFRGLENPLFPKERFNDLIS
CIRIDNLYERALHIRKLLLTLP RSVLIVMRYLFAFLNHLSQYSDENMMD
PYNLAICFGPTLMPVPEIQDQVSCQAHVNEIKTI I IHHETIFPDAKEL
DGPVYEKCMAGDDYCDSPYSEHGTLEEVDQDAGTEPHTSEDECEPIEAI
AKFDYVGRSARELSFKKGASLLLYHRASEDWWEGRHNGIDGLVPHQYIV
VQDMDDTFSDTLSQKADSEASSGPVTE DKSSSKDMNSPTDRHPDGYLAR
QRKRGEPPPVR RPGR TSDGH C PLHPPHALSNSSVDLGSPSLASHPRGL
LQNRGLNNDSPERRRRPGHGS LTNISRHDSLKKIDSPPIRRSTSSGQYT
GFNDHKPLDPETIAQDIEETMNTALNELRELERQSTAKHAPDVVLDTLE
QVKNSPTPATSTESLSPLHNVALRSSEPQIRRSTSSSSDTMSTFKPMVA
PRMGVQLKPPALRPKPAVL PKTNPTIGPAPPPQGPTDKSCTM

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Native sequence Amino acids M1 – M1085 (end) of human srGAP1.
Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *Bam*H1 and *Not*1 sites of pGEX6P1

Nucleotide Sequence of Insert:

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ggatccATGTCCACCCCGAGCCGATTCAAGAAGGACAAAGAGATCATAGC
CGAGTATGAAAGTCAAGTCAAAGAAATTCGAGCTCAACTGGTAGAACAAAC
AAAAATGCCTGGAGCAGCAAACGGAGATGCGAGTTCAGCTTCTCCAGGAT
CTGCAAGATTTCTTCCGAAAAAAGCTGAAATTGAGACGGAATATTCCTCG
GAATCTAGAGAAGTTAGCAGAAAGGTTTCATGGCAAAAACAAGAAGCACTA
AGGATCATCAACAATACAAGAAAGACCAGAACCTGTTGTCTCCAGTGAAC
TGCTGGTATTTGCTCCTGAACCAAGTAAGGAGAGAAAGCAAAGACCATGC
AACCTTGAGTGACATCTATCTGAACAATGTGATTATGCGGTTTCATGCAGA
TAAGTGAGGATTCTACCAGGATGTTTAAAAAGAGCAAAGAGATTGCATTC
CAACTTCATGAGGATTTAATGAAGGTTCTTAATGAGCTTTTATACGGTGAT
GAAAACATACCATATGTATCATGCAGAGAGCATCAGTGCAGAGAGCAAGC
TGAAAGAGGGCCGAAAAACAAGAGGAAAAGCAAATTGGGAGATCTGGTGAT
CCAGTCTTCCATATTCGACTAGAGGAGAGACATCAACGGCGAAGCTCTGT
AAAGAAAATTGAAAAATGAAAGAAAAAGACAAGCAAATATTCAGAAA
ATAAGCTAAAATCAATTAAGGCACGGAACGAATATCTCCTAACACTTGAA
GCCACCAATGCCCTCAGTTTTCAAGTACTATATTCATGATCTTTCTGATTT
AATTGATTGCTGTGATCTTGGCTACCATGCAAGTCTGAACAGAGCCCTAA
GAACATATCTGTCTGCGGAGTACAACCTTGAAACCTCCAGACATGAGGGC
TTAGACATTATTGAGAATGCAGTTGATAATTTAGAGCCCAGGAGCGATAA
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CAGCCAGTCCAGGCAGAGCTCATGCTCAGGTACCAACAGTTGCAGTCCCG
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TCTGAATGCTTCCAGCACAGTCGTTCCACAGAATCTGTGAAGTCCACTGT
CTCTGAAACCTACCTGAGTAAACCCAGCATCGCCAAGAGAAGAGCCAACC
AGCAGGAAACTGAACAGTTCTACTTCATGAAACTCAGAGAATATTTGGAA
GGCAGTAATCTCATCACAAAACCTTCAAGCCAAACATGACTTGCTGCAGAG
GACCCTGGGAGAAGGTCATAGAGCTGAATATATGACTACAAGGCCTCCAA
ATGTTCCCCCTAAGCCCCAGAAACACAGGAAGTCCAGGCCCGCTCACAG
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TCTATGGTCTTCAGCATCAGGGGATTTTCAGAGTGTCTGGTTCAGGTTG
GAAGTCAATGATATTAATAATTCATTTGAGAGAGGTGAAAATCCTTTGGC
TGATGACCAGAGTAACCATGATATTAACCTCAGTTGCTGGCGTTCTGAAGC
TCTATTTCCGTGGGCTGGAAAACCCCTCTTTCCTAAGGAAAGATTTAAC
GATCTGATTTCTTGTATCAGAAATAGATAATCTCTATGAGAGGGCGCTTCA
CATCCGCAAACCTCCTCCTGACTTTGCCAGGTCGGTCCTTATAGTGATGA
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GGTACCTCTTTGCCTTCCTCAATCATCTATCACAGTACAGCGATGAGAAT
ATGATGGACCCTTATAACCTGGCCATTTGCTTTGGCCCAACATTGATGCC
TGTCCCAGAAATACAGGATCAAGTGTCTTGCCAGGCACATGTGAATGAAA
TTATCAAAAACCATCATCATCCACCATGAGACTATTTTCCCAGATGCTAAA
GAGCTGGATGGCCCTGTTTATGAGAAAATGTATGGCTGGAGATGACTATTG
CGACAGCCCATACAGTGAGCACGGTACATTGGAGGAAGTGGACCAAGATG
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ATAGCCAAGTTTGACTATGTTGGGCGGTCCGCCAGAGAACTATCCTTCAA
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TGGCAGCCTGACCAACATCAGCCGGCAGACTCCCTCAAGAAGATCGACA
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GACCACAAGCCACTGGACCCAGAGACAATTGCTCAGGATATTGAAGAAAC
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CAAAGCATGCCCCTGATGTGGTGTGGATACCCTGGAGCAAGTGAAAAAC
TCTCCCACCCCTGCCACTTCCACGGAATCTCTCAGCCCTTTGCACAACGT
TGCCCTCAGGAGCTCCGAGCCTCAGATTCGACGTAGCACGAGCTCCTCCA
GTGACACAATGAGTACTTTCAAGCCTATGGTGGCACCCAGAATGGGCGTG
CAGCTGAAGCCTCCAGCCCTTAGGCCAAAACCTGCTGTTCTTCCAAAAAC
AAATCCTACCATAGGACCTGCCCCACCTCCCAGGGTCCAACAGACAAGT
CATGCACAATGTAAgcggccgc