

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of srGAP2A [1 – 1071]**

**Enzyme description:-** srGAP2A [1 – 1071]

**Clone number:-** DU 11367

**Source:-** Recombinant

**Expression system:-** *E.coli*

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 148, 651.79 daltons

Average Mass 148, 745.88 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 6.15

**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**

**srGAP2A [1 – 1071]**

<b><u>Protein</u></b>	srGAP2A [1 – 1071]
<b><u>Clone number</u></b>	DU 11367
<b><u>Species</u></b>	Human
<b><u>Accession number</u></b>	NM_015326.4
<b><u>Tags</u></b>	N-terminal GST
<b><u>Bacterially expressed protein</u></b>	<p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEELHYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIESMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFOGPLGSPEIPGSTRAAAMTS <b>PAKFKKDKEI IAEYDTQVKEIRAQLTEQMKCLDQOQCELRVQLLQDLQDF</b> <b>FRKKAIEIMDYSRNLEKLAERFLAKTRSTKDQQFKKDQNVLSPVNCWNL</b> <b>LLNQVKRESRDHTTSLSDIYLNNIIPRFVQVSEDSGRLFKKSKEVGOQLO</b> <b>DDLKVLNELYSVMKTYHMYNADSI SAQSKLKEAEKQEEKQIGKSVKQE</b> <b>DRQTPRSPDSTANVRIEEKHVRRSSVKKIEKMKEKRQAKYTENKLKAIK</b> <b>ARNEYLLALEATNASVFKYYIHDLSDLIDQCCDLGYHASLNRALRTFLS</b> <b>AELNLEQSKHEGLDAIENAVENLDATSDKQRLMEMYNNVFCPPMKFEFQ</b> <b>PHMGDMASQLCAQQPVQSELVQRCQQLQSRLSTLKIENEVKKTMEATL</b> <b>QTIQDIVTVEDFDVSDCFQYSNSMESVKSTVSETFMSKPSIAKRRANQQ</b> <b>ETEQFYFTKMKEYLEGRNLITKLQAKHDLLOKTLGESQRTDCSLARRSS</b> <b>TVRKQDSSQAIPLVVESCIRFISRHGLQHEGIFRVSGSQVEVNDIKNAF</b> <b>ERGEDPLAGDQNDHDMDSIAGVLKLYFRGLEHPLFPKDFHDLMACVTM</b> <b>DNLQERALHIRKVLLVLPKTTLIIMRYLFAFLNHLSQFSEENMMDPYNL</b> <b>AICFGPSLMSVPEGHDQVSCQAHVNELIKTIIIQHENIFPSPRELEGPV</b> <b>YSRGGSMEDYCDSPHGETTSVEDSTQDVTAEHHTSDDECEPIEAIKFD</b> <b>YVGR TARELSFKKGASLLLYQRASDDWWEGRHNGIDGLIPHQYIVVQDT</b> <b>EDGVVERS SPKSEIEVISEPPEEKVTARAGASCPGGHVADIYLANINK</b> <b>QRKRPESGSIRKTRFRSDSHGLSSSLTDSSSPGVGASCRPSSQPIMSQSL</b> <b>PKEGPDKCSISGHGSLNSISRHSSLKNRLDSPQIRKTATAGRSKSFNNH</b> <b>RPMDPEVIAQDIEATMNSALNELRELERQSSVKHTPDVVLDTLEPLKTS</b> <b>PVVAPTSEPSSPLHTQLLKDPEPAFORSASTAGDIACAFRPVKSVMKMAA</b> <b>PVKPPATRPKPTVFPKTNATSPGVNSSTSPQSTDKSCTV</b></p>

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**Native sequence**      Amino acids M1 – V1071 (end) of human srGAP2A.  
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 (LEVLFQGP) residues 221 - 228

**Cloning sites**          *Not1* sites of pGEX6P2

### **Nucleotide Sequence of Insert:**

```
gcggccgcgATGACGTCTCCAGCCAAATTCAAAAAGGATAAGGAGATCAT
AGCAGAGTACGATACTCAGGTCAAAGAGATCCGTGCTCAGCTCACAGAGC
AGATGAAATGCCTGGACCAGCAGTGTGAGCTTCGGGTGCAACTGTTGCAG
GACCTCCAGGACTTCTTCCGAAAGAAGGCAGAGATTGAGATGGACTACTC
CCGCAACCTGGAGAAGCTGGCAGAACGCTTCCCTGGCCAAGACACGCAGCA
CCAAGGACCAGCAATTCAGAAGGATCAGAATGTTCTCTCTCCAGTCAAC
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GCTCTGCACATCCGGAAAGTCCTCCTAGTCCTGCCCAAGACCACTCTGAT  
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