

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of PROTOR1 [1 – 388]**

**Enzyme description:-** PROTOR1 [1 - 388]

**Clone number:-** DU 10413

**Source:-** Recombinant

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

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 69, 533.56 daltons

Average Mass 69, 577.64 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 6.59

**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**

**PROTOR1 [1 - 388]**

<b><u>Protein</u></b>	PROTOR1 [1 - 388]
<b><u>Clone number</u></b>	DU 10413
<b><u>Species</u></b>	Human
<b><u>Accession number</u></b>	NM_181333.3
<b><u>Tags</u></b>	N-terminal GST
<b><u>Bacterially expressed protein</u></b>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSMRTLRRLLKFMSSPS <b>LSDLGKREPAAAADERGTQORRACANATWNSIHNGVIAVFQRKGLPDQE</b> <b>LFSLNEGVRQLLKTTELGSFFTEYLQNQLLTKGMVILRDKIRFYEGOKLL</b> <b>DSLAEWDFFFSDVLPMLQAIIFYPVQGKEPSVRQLALLHFRNAITLSVK</b> <b>LEDALARAHARVPPAIVQMLLVLQGVHESRGVTELYLRLETLVQKVVSP</b> <b>YLGTYGLHSSEGPFTHSCILEKRLRRSRSGDVLAKNPVVRSSKSYNTPL</b> <b>LNPVQEHAEAGAAAGGTSIRRHSVSEMTSCPEPQGFSDPPGQGPQTGTFR</b> <b>SSPAPHSGPCPSRLYPTTQPPEQGLDPTRSSLPSSPENLVDQILESVD</b> <b>SDSEGIFFIDFGRGRGSGMSDLEGSGRQSVV</b></p>
<b><u>Native sequence</u></b>	<p>Amino acids M1 – V388 (end) of human PROTOR1. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<b><u>Protease cleavage</u></b>	PreScission ( <u>LEVLFGQP</u> ) residues 221 – 228
<b><u>Cloning sites</u></b>	<i>Bgl</i> 2 and <i>Not</i> 1 sites of pGEX6P-1

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Nucleotide Sequence of insert

agatctATGAGGACTCTCCGCAGGTTGAAGTTCATGAGTTCGCCCAGCCTCAGTGACCTGGGCAAG  
AGAGAGCCGGCCGCCGCCGCGGACGAGCGGGGCACGCAGCAGCGCCGGGCCTGCGCCAACGCCACC  
TGGAACAGCATCCACAACGGGGTGATCGCCGTCTTCCAGCGCAAGGGGCTGCCCGACCAGGAGCTC  
TTCAGCCTCAACGAGGGCGTCCGGCAGCTGTTGAAGACAGAGCTGGGGTCTTCTTTCACGGAGTAC  
CTGCAGAACCAGCTGCTGACAAAAGGCATGGTGATCCTTTCGGGACAAGATTCGCTTCTATGAGGGA  
CAGAAGCTGCTGGACTCACTGGCAGAGACCTGGGACTTCTTCTTTCAGTGACGTGCTGCCCATGCTG  
CAGGCCATCTTCTACCCGGTGCAGGGCAAGGAGCCATCGGTGCGCCAGCTGGCCCTGCTGCACTTC  
CGGAATGCCATCACCCCTCAGTGTGAAGCTAGAGGATGCGCTGGCCCGGGCCCATGCCCGTGTGCC  
CCTGCCATCGTGAGATGCTGCTGGTGCTGCAGGGGTACATGAGTCCAGGGGCGTGACTGAGGAC  
TACCTGCGCCTGGAGACGCTGGTCCAGAAGGTGGTGTGCGCCATACCTGGGCACGTACGGCCTCCAC  
TCCAGCGAGGGGCCCTTACCCATTCTGCATCCTGGAAAAGCGCCTCCTCCGCCGCTCCCGCTCG  
GGGACGTGCTGGCCAAGAACCCTGTGGTGCGCTCCAAGAGCTACAACACGCCTCTGCTGAACCC  
GTGCAGGAGCACGAGGCGGAGGGCGCGGCCGGCGGTACCAGCATCCGCAGGCACTCTGTGTGCG  
GAGATGACGTCTTGCCTCCCGAGCCTCAGGGCTTCTCCGACCCGCCCGGCCAGGGCCCCACCGGACC  
TTCAGGTCTTCCCGGCCGCCCACTCAGGGCCCTGCCCCAGCAGACTGTACCCACGACCCAGCCC  
CCTGAGCAGGGCTTGGATCCCACCCGAGCTCCTTGCCTCCAGCCCGGAGAACCTGGTGGAC  
CAGATCCTGGAGTCCGTGGACTCGGATTTCTGAAGGGATTTTCATTGACTTTGGCCGGGGCCGGGGC  
TCTGGCATGTCCGACTTGGAGGGCTCTGGGGGCCGGCAGAGTGTTCGTGtgagcggccgc