

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of PI31 [1 – 271]**

<b><u>Enzyme description:-</u></b>	PI31 [1 - 271]
<b><u>Clone number:-</u></b>	DU 34918
<b><u>Source:-</u></b>	Recombinant
<b><u>Expression system:-</u></b>	<i>E.coli</i>
<b><u>Tag:-</u></b>	N-terminal GST
<b><u>Purification method:-</u></b>	GSH Sepharose

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

Monoisotopic      57, 574.06 daltons  
Average Mass      57, 611.02 daltons  
[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-**                      5.54

**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

#### PI31 [1 - 271]

<u>Protein</u>	PI31 [1 - 271]
<u>Clone number</u>	DU 34918
<u>Species</u>	Human
<u>Accession number</u>	D88378.1
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLVLFQGPLGSPEFALMHTSMAGLE <b>VLFASAAPAITCRQDALVCFHWEVVTHGYCGLGVDQPGPNPKSELL</b> <b>PAGWNNNKDLYVLRYEYKDGSRKLLVKAITVESSMILNVLEYGSQQVAD</b> <b>LTLNDDYIDAHLGDFHRTYKNSEELRSRIVSGIITPIHEQWEKANVS</b> <b>SPHREFPPATAREVDPLRIPPHHPHTSRQPPWCDPLGPFVVGGEDLDPF</b> <b>GPRRGMIVDPLRSGFPRALIDPSSGLPNRLPPGAVPPGARFDPFGPIG</b> <b>TSPPGPNPDHLPPPGYDDMYL</b></p>
<u>Native sequence</u>	<p>Amino acids M1 – L271 (end) of human PI31. Residue M241 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<u>Protease cleavage</u>	PreScission ( <u>LEVLFQGP</u> ) residues 221 - 228
<u>Cloning sites</u>	<i>Spe</i> 1 and <i>Not</i> 1 sites of pGEX6P-1

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Nucleotide Sequence of insert

ggatccccggaattcgcccttATGCATACTAGTATGGCGGGCCTGGAGGTACTGTTTCGCATCGGCA  
GCGCCGGCCATCACCTGCAGGCAGGACGCGCTCGTCTGCTTCTTGCATTGGGAAGTGGTGACACAC  
GGTTACTGCGGCTTGGGTGTCGGTGACCAGCCGGGTCCCAATGATAAGAAGTCAGAACTGCTGCCA  
GCTGGGTGGAACAACAATAAAGACCTGTATGTCCTCCGGTATGAGTATAAGGATGGGTCCAGAAAG  
CTCCTTGTGAAAGCCATCACCGTGGAGAGCAGCATGATCCTCAATGTGCTGGAATATGGCTCACAG  
CAAGTGGCAGACTTGACCCTGAACTTGGATGATTATATCGATGCAGAACACCTGGGTGACTTCCAC  
AGGACCTACAAGAACAGTGAGGAGCTTCGGTCTCGTATTGTGTCTGGAATCATCACACCTATCCAT  
GAGCAGTGGGAAAAGGCTAATGTAAGCAGTCCCCACCGGGAGTCCCCCTGCTACCGCCAGAGAG  
GTGGACCCACTCCGGATTCCTCCACACCACCCACACACCAGTCGGCAGCCTCCCTGGTGTGATCCC  
CTGGGCCCGTTTGTGTCGGGGGAGAAGACTTAGACCCTTTTGGGCCCTCGGAGAGGTGGCATGATT  
GTGGATCCCCTGAGATCTGGCTTCCCAAGAGCACTTATTGACCCTTCCTCAGGCCTCCCGAACCGA  
CTTCCTCCAGGCGCTGTGCCCCCAGGAGCTCGCTTTGACCCCTTTGGACCCATTGGGACCAGCCCA  
CCCGGACCTAACCCAGACCATCTCCCCCGCCGGGCTACGATGACATGTACCTGtgagcggcgc

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