

## *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 38507
<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, 465.93 daltons  
Average Mass      57, 502.74 daltons  
[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-**                      5.35

**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]**

<b><u>Protein</u></b>	PI31 [1 - 271]
<b><u>Clone number</u></b>	DU 38507
<b><u>Species</u></b>	Mouse
<b><u>Accession number</u></b>	NM_212446.1
<b><u>Tags</u></b>	N-terminal GST
<b><u>Bacterially expressed protein</u></b>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLVLFQGPLGSPEFALMHTSMAGLE <b>VLFAAAPSMSCPQDALVCFHWEVVTNGYYALGTGDQPGPSDKKSELL</b> <b>PAKWNSNKELYALRYESKDGARKLLLKAVSVENGMIINVLELGTQOVAD</b> <b>LTLNDDYIDAEDLSDFHRTYKNSEELRSQIRSGIITPIHEQWEKARAN</b> <b>SPPREFPPATAREVDPLQISSHRPHTSRQPAWRDPLSPFAVGGDDLDPF</b> <b>GCQRGGMIVDPLRSGFPRVLIDPSSGLPNRLPPGAVPPGARFDPFGPIG</b> <b>TSPSGPNPDHLPPPGYDDMYL</b></p>
<b><u>Native sequence</u></b>	<p>Amino acids M1 – L271 (end) of mouse 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>
<b><u>Protease cleavage</u></b>	PreScission ( <u>LEVLFQGP</u> ) residues 221 - 228
<b><u>Cloning sites</u></b>	<i>Spe</i> 1 and <i>Not</i> 1 sites of pGEX6P-1

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### Nucleotide Sequence of insert

ggatccccggaattcgcccttATGCATACTAGATGGCGGGTCTCGAGGTTCTCTTCGCGTCGGCCG  
CTCCATCCATGAGCTGCCCCGAGGACGCACTCGTCTGCTTCTTGCCTGGGAAGTGGTGACAAACG  
GCTACTATGCCTTGGGCACGGGCGACCAGCCAGGTCCAGTGATAAGAAATCAGAACTACTGCCAG  
CTAAGTGGAACAGCAATAAAGAACTGTATGCCCTCCGGTATGAGTCTAAGGATGGAGCCAGAAAAC  
TCCTTCTGAAAGCTGTCTCTGTGGAGAACGGCATGATCATCAACGTGCTGGAACCTGGCACACAGC  
AGGTGGCAGACTTGACCCTGAACTTAGATGATTATATTGATGCAGAAGACCTGAGTGACTTCCACA  
GGACCTATAAGAACAGTGAGGAGCTTCGGTCTCAGATCAGATCTGGAATTATCACACCTATCCATG  
AGCAGTGGGAGAAGGCTCGTGCAAACAGTCTCCGAGGGAGTCCCACCTGCCACTGCCAGAGAGG  
TGGACCCACTCCAGATTCCTCACACCGTCTCATACTAGCAGGCAGCCTGCTTGGCGTGATCCCC  
TGAGCCCATTTGCTGTCGGGGGAGATGACCTAGACCCTTTTGGGTGTCAGAGAGGTGGCATGATTG  
TGGATCCCCTGAGATCCGGCTTCCCAAGAGTACTTATTGACCCATCCTCAGGCCTCCCAAACCGGC  
TTCCTCCTGGTGCTGTGCCCCCAGGAGCTCGCTTTGACCCCTTTGGACCCATTGGGACCAGCCCAT  
CTGGACCTAACCCCTGACCATCTTCCCCACCGGGCTACGATGACATGTACCTGtgagcggccgc

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