

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of Optineurin [1 - 577]**

**Enzyme description:-** Optineurin [1 – 577]

**Clone number:-** DU 4637

**Source:-** Recombinant

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

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 92, 687.81 daltons

Average Mass 92, 746.21 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 5.23

**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**

**Optineurin [1 – 577]**

<b><u>Protein</u></b>	Optineurin [1 – 577]
<b><u>Clone number</u></b>	DU 4637
<b><u>Species</u></b>	Human
<b><u>Accession number</u></b>	AF420371
<b><u>Tags</u></b>	N-terminal GST
<b><u>Bacterially expressed protein</u></b>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPKSDLEVLFGQPLGSMHQPLSCLTEKED <b>SPSESTGNGPPHLAHPNLDFTTPEELLQOMKELLTENHQLKEAMKLNNQ</b> <b>AMKGRFEELSAWTEKQKEERQFFEIQSKEAKERLMALSHENEKLKEELG</b> <b>KLKGKSERSSDPTDDSRLPRAEAEQEKDQLRTQVVRLOAEKADLLGIV</b> <b>SELQLKLNSSGSSSEDSFVEIRMAEGEAEGSVKEIKHSPGPTRTVSTGTA</b> <b>LSKYRSRSADGAKNYFEHEELTVSOLLCLREGNQKVERLEVALKEAKE</b> <b>RVSDFEKKTNRSEIETQTEGSTEKENDEEKGPETVGSEVEALNLQVTS</b> <b>LFKELQEAHTKLSEAELMKKRLQEKQALERKNSAIPSELNEKQELVYT</b> <b>NKKLELQVESMLSEIKMEQAKTEDEKSKLTVLQOMTHNKLLQEHNNALKT</b> <b>IEELTRKESEKVDRAVLKELSEKLELAEKALASKQLQMDQEMKQTIKQE</b> <b>EDLETMTILRAQMEVYCSDFHAERAAREKIHEEKEQLALQLAVLLKEND</b> <b>AFEDGGRQSLMEMQSRHGARTSDSDQAYLVQVQGAEDRDWRQQRNIPIH</b> <b>SCPCKGEVLPDIDTLQIHVMDCII</b></p>
<b><u>Native sequence</u></b>	Amino acids M1 – I577 (end) of human Optineurin. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
<b><u>Protease cleavage</u></b>	PreScission ( <u>LEVLFGQP</u> ) residues 221 - 228
<b><u>Cloning sites</u></b>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pGEX6P-1

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### Complete Nucleotide Sequence:

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCAC  
TCGACTTCTTTTGGAAATATCTTGAAGAAAAATATGAAGAGCATTGTATG  
AGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGTTTG  
GAGTTTCCCAATCTTCCATTATTATATTGATGGTGATGTTAAATTAACACA  
GTCTATGGCCATCATACTTATATAGCTGACAAGCACAACATGTTGGGTG  
GTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTG  
GATATTAGATACGGTGTTCGAGAATTGCATATAGTAAAGACTTTGAAAC  
TCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTTCG  
AAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCATGTAACCCAT  
CCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATAACATGGACCC  
AATGTGCCTGGATGCGTTCCTCAAATTAGTTTTGTTTTAAAAACGTATTG  
AAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGCAAGTATATAGCA  
TGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCC  
AAAATCGGATCTGGAAGTTCTGTTCCAGGGGCCCTGGGATCCATGTCCC  
ATCAACCTCTCAGCTGCCTCACTGAAAAGGAGGACAGCCCCAGTGAAGC  
ACAGGAAATGGACCCCCCACCTGGCCACCCAAACCTGGACACGTTTAC  
CCCGGAGGAGCTGCTGCAGCAGATGAAAGAGCTCCTGACCGAGAACCACC  
AGCTGAAAGAAGCCATGAAGCTAAATAATCAAGCCATGAAAGGGAGATTT  
GAGGAGCTTTTCGGCCTGGACAGAGAAACAGAAGGAAGAACGCCAGTTTTT  
TGAGATACAGAGCAAAGAAGCAAAAGAGCGTCTAATGGCCTTGAGTCATG  
AGAATGAGAAATTGAAGGAAGAGCTTGGAAAATAAAAGGGAAATCAGAA  
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AGAAGGCAGACCTGTTGGGCATCGTGTCTGAACTGCAGCTCAAGCTGAAC  
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AGAAGCAGAAGGGTCAAGTAAAAGAAATCAAGCATAGTCTGGGCCACGA  
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CCTGCTGTGCCTAAGGGAAGGGAATCAGAAGGTGGAGAGACTTGAAGTTG  
CACTCAAGGAGGCCAAAAGAAAGAGTTTTAGATTTTGAAGAAAACAAGT  
AATCGTTCTGAGATTGAAACCCAGACAGAGGGGAGCACAGAGAAAGAGAA  
TGATGAAGAGAAAGGCCCGGAGACTGTTGGAAGCGAAGTGGAAAGCACTGA  
ACCTCCAGGTGACATCTCTGTTTAAAGGAGCTTCAAGAGGCTCATACAAAA  
CTCAGCGAAGCTGAGCTAATGAAGAAGAGACTTCAAGAAAAGTGTGAGGC  
CCTTGAAGGAAAAATCTGCAATTCCATCAGAGTTGAATGAAAAGCAAG  
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TCAGAAATCAAATGGAACAGGCTAAAACAGAGGATGAAAAGTCCAAATT  
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CATTGAAAACAATTGAGGAACTAACAGAAAAGAGTCAGAAAAAGTGGAC  
AGGGCAGTGTGAAGGAACTGAGTGAAGAACTGGAAGTGGCAGAGAAGGC  
TCTGGCTTCCAAACAGCTGCAAATGGATGAAATGAAGCAAACCATTGCCA  
AGCAGGAAGAGGACCTGGAAACCATGACCATCCTCAGGGCTCAGATGGAA  
GTTTACTGTTCTGATTTTCATGCTGAAAGAGCAGCGAGAGAGAAAATTC

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TGAGGAAAAGGAGCAACTGGCATTGCAGCTGGCAGTTCTGCTGAAAGAGA  
ATGATGCTTTCGAAGACGGAGGCAGGCAGTCCTTGATGGAGATGCAGAGT  
CGTCATGGGGCGAGAACAAGTGACTCTGACCAGCAGGCTTACCTTGTTCA  
AAGAGGAGCTGAGGACAGGGACTGGCGGCAACAGCGGAATATTCGGATTC  
ATTCCTGCCCCAAGTGTGGAGAGGTTCTGCCTGACATAGACACGTTACAG  
ATTCACGTGATGGATTGCATCATTtaagcggccgc