

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of Optineurin E478A [1 - 577]

Enzyme description:- Optineurin E478A [1 – 577]

Clone number:- DU 4651

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 92, 629.80 daltons

Average Mass 92, 688.18 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.25

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 E478A [1 – 577]

<u>Protein</u>	Optineurin E478A [1 – 577]
<u>Clone number</u>	DU 4651
<u>Species</u>	Human
<u>Accession number</u>	AF420371
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPKSDLEVLFGQPLGSMHQPLSCLTEKED SPSESTGNGPPHLAHPNLDFTTPEELLQOMKELLTENHQLKEAMKLNNQ AMKGRFEELSAWTEKQKEERQFFEIQSKEAKERLMALSHENEKLKEELG KLKGKSERSSDPTDDSRLLPRAEAEQEKDQLRTOVVRLQAEKADLLGIV SELQKLNSSGSSSEDSFVEIRMAEGEAEGSVKEIKHSPGPTRTVSTGTA LSKYRSRSADGAKNYFEHEELTVSOLLCLREGNQKVERLEVALKEAKE RVSDFEKKTNRSEIETQTEGSTEKENDEEKGPETVGSEVEALNLQVTS LFKELQEAHTKLSEAELMKKRLQEKQALERKNSAIPSELNEKQELVYT NKKLELQVESMLSEIKMEQAKTEDEKSKLTVLQMTNKLLOEHNALKT IEELTRKESEKVDRAVLKELSEKLELAEKALASKQLQMDKQTIKQE EDLETMTILRAQMEVYCSDFHARAAREKIHEEKEQLALQLAVLLKEND AFEDGGRQSLMEMQSRHGARTSDSDQQAYLVQGAEDRDWRQQRNIPIH SCPKCGEVLPDIDTLQIHVMDCII</p>
<u>Native sequence</u>	<p>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. The protein has an E478A mutation. Residue E478 is equivalent to residue A709 of the fusion protein.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pGEX6P-1

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Complete Nucleotide Sequence:

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCAC
TCGACTTCTTTTGAATATCTTGAAGAAAAATATGAAGAGCATTGTATG
AGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGTTTG
GAGTTTCCCAATCTTCTTATTATATTGATGGTGATGTTAAATTAACACA
GTCTATGGCCATCATACTTATATAGCTGACAAGCACACATGTTGGGTG
GTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTG
GATATTAGATACGGTGTTCGAGAATTGCATATAGTAAAGACTTTGAAAC
TCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTCG
AAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCATGTAACCCAT
CCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATAACATGGACCC
AATGTGCCTGGATGCGTTCCTAAAATTAGTTTGTTTTAAAAAACGTATTG
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TGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCC
AAAATCGGATCTGGAAGTTCTGTTCCAGGGGCCCTGGGATCCATGTCCC
ATCAACCTCTCAGCTGCCTCACTGAAAAGGAGGACAGCCCCAGTGAAAGC
ACAGGAAATGGACCCCCCACCTGGCCACCCAAACCTGGACACGTTTAC
CCCGGAGGAGCTGCTGCAGCAGATGAAAGAGCTCCTGACCGAGAACCACC
AGCTGAAAGAAGCCATGAAGCTAAATAATCAAGCCATGAAAGGGAGATTT
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AGGTCATCTGAGGACCCCACTGATGACTCCAGGCTTCCCAGGGCCGAAGC
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CCTGCTGTGCCTAAGGGAAGGGAATCAGAAGGTGGAGAGACTTGAAGTTG
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TGAGGAAAAGGAGCAACTGGCATTGCAGCTGGCAGTTCTGCTGAAAGAGA
ATGATGCTTTCGAAGACGGAGGCAGGCAGTCCCTTGATGGAGATGCAGAGT

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CGTCATGGGGCGAGAACAAGTGACTCTGACCAGCAGGCTTACCTTGTTCA
AAGAGGAGCTGAGGACAGGGACTGGCGGCAACAGCGGAATATTCGGATTC
ATTCCTGCCCCAAGTGTGGAGAGGTTCTGCCTGACATAGACACGTTACAG
ATTCACGTGATGGATTGCATCATTtaagcggccgc