

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of Optineurin E50K D474N [1 - 577]

Enzyme description:- Optineurin E50K D474N [1 – 577]

Clone number:- DU 4825

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 92, 685.88 daltons

Average Mass 92, 744.29 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.30

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 E50K D474N [1 – 577]

<u>Protein</u>	Optineurin E50K D474N [1 – 577]
<u>Clone number</u>	DU 4825
<u>Species</u>	Human
<u>Accession number</u>	AF420371
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPKSDLEVLFGQPLGSMHQPLSCLTEKED SPSESTGNGPPHLAHPNLDFTTPEELLQOMKELLTKNHQLKEAMKLNNQ AMKGRFEELSAWTEKQKEERQFFEIQSKEAKERLMALSHENEKLKEELG KLKGKSERSSDPTDDSRLPRAEAEQEKDQLRTOVVRLQAEKADLLGIV SELQKLNSSGSSSEDSFVEIRMAEGEAEGSVKEIKHSPGPTRTVSTGTA LSKYRSRSADGAKNYFEHEELTVSOLLCLREGNQKVERLEVALKEAKE RVSDFEKTSNRSEIETQTEGSTEKENDEEKGPETVGSEVEALNLQVTS LFKELQEAHTKLSEAELMKKRLQEKQALERKNSAIPSELNEKQELVYT NKKLELQVESMLSEIKMEQAKTEDEKSKLTVLQMTNKLLOEHNNALKT IEELTRKESEKVDRAVLKELSEKLELAEKALASKQLQMDKQTIQAKQE EDLETMTILRAQMEVYCSNFHAERAAREKIHEEKEQLALQLAVLLKEND 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 E50K mutation and a D474N mutation. Residue E50 is equivalent to residue K281 of the fusion protein. And residue D474 is equivalent to residue N705 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
TCGACTTCTTTTGGAAATATCTTGAAGAAAAATATGAAGAGCATTTGTATG
AGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGTTTG
GAGTTTCCCAATCTTCTTATTATATTGATGGTGATGTTAAATTAACACA
GTCTATGGCCATCATACTGTTATATAGCTGACAAGCACAAACATGTTGGGTG
GTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTG
GATATTAGATACGGTGTTCGAGAATTGCATATAGTAAAGACTTTGAAAC
TCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTTCG
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CCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATAACATGGACCC
AATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAAAAACGTATTG
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TGCCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCC
AAAATCGGATCTGGAAGTTCTGTTCCAGGGCCCTGGGATCCATGTCCC
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ACAGGAAATGGACCCCCCACCTGGCCACCCAAACCTGGACACGTTTAC
CCCGGAGGAGCTGCTGCAGCAGATGAAAGAGCTCCTGACCAAGAACCACC
AGCTGAAAGAAGCCATGAAGCTAAATAATCAAGCCATGAAAGGGAGATTT
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AGGTCATCTGAGGACCCCACTGATGACTCCAGGCTTCCAGGGCCGAAGC
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AGAAGGCAGACCTGTTGGGCATCGTGTCTGAACTGCAGCTCAAGCTGAAC
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CCTGCTGTGCCTAAGGGAAGGGAATCAGAAGGTGGAGAGACTTGAAGTTG
CACTCAAGGAGGCCAAAGAAAGAGTTTCAGATTTTGAAAAGAAAACAAGT
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TGATGAAGAGAAAGGCCCGGAGACTGTTGGAAGCGAAGTGGAAGCACTGA
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ATGATGCTTTCGAAGACGGAGGCAGGCAGTCCCTTGATGGAGATGCAGAGT
CGTCATGGGGCGAGAACAAGTGACTCTGACCAGCAGGCTTACCTTGTTC

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AAGAGGAGCTGAGGACAGGGACTGGCGGCAACAGCGGAATATTCGGATTC
ATTCCTGCCCCAAGTGTGGAGAGGTTCTGCCTGACATAGACACGTTACAG
ATTCACGTGATGGATTGCATCATTtaagcggccgc