Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of Optineurin E50K [1 - 577]

Enzyme description:- Optineurin E50K [1 – 577]

Clone number:- DU 8952

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 92, 686.86 daltons
Average Mass 92, 745.27 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.27

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 [1 – 577]

Protein Optineurin E50K [1 – 577]

Clone number DU 8952

Species Human

Accession number AF420371

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA
VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MSHQPLSCLTEKED**
SPSESTGNGPPHLAHPNLDTFTPEELLQQMKELLTKNHQLKEAMKLNNQ
AMKGRFEELSAWTEKQKEERQFFEIQSKEAKERLMALSHENEKLKEELG
KLKGKSERSSEDPTDDSRLPRAEAEQEKDQLRTQVVRLQAEKADLLGIV
SELQLKLNSSGSSEDSFVEIRMAEGEAEGSVKEIKHSPGPTRTVSTGTA
LSKYRSRSADGAKNYFEHEELTVSQLLLCLREGNQKVERLEVALKEAKE
RVSDFEKKTSNRSEIETQTEGSTEKENDEEKGPETVGSEVEALNLQVTS
LFKELQEAHTKLSEAELMKKRLQEKCQALERKNSAIPSELNEKQELVYT
NKKLELQVESMLSEIKMEQAKTEDEKSKLTVLQMTHNKLLQEHNNALKT
IEELTRKESEKVDRAVLKELSEKLELAEKALASKQLQMDEMKQTIAKQE
EDLETMTILRAQMEVYCSDFHAERAAREKIHEEKEQLALQLAVLLKEND
AFEDGGRQSLMEMQSRHGARTSDSDQQAYLVQRGAEDRDWRQQRNIPIH
SCPKCGEVLPDIDTLQIHVMDCII

Native sequence 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. Residue E50 is equivalent to residue K281 of the fusion protein.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *Bam*H1 and *Not*1 sites of pGEX6P-1

Nucleotide Sequence Of Insert:

ggatccATGTCCCATCAACCTCTCAGCTGCCTCACTGAAAAGGAGGACAG
CCCCAGTGAAAGCACAGGAAATGGACCCCCCCACCTGGCCCACCCAAACC
TGGACACGTTTACCCCGGAGGAGCTGCTGCAGCAGATGAAAGAGCTCCTG
ACCAAGAACCACCAGCTGAAAGAAGCCATGAAGCTAAATAATCAAGCCAT
GAAAGGGAGATTTGAGGAGCTTTCGGCCTGGACAGAGAAACAGAAGGAAG
AACGCCAGTTTTTTGAGATACAGAGCAAAGAAGCAAAAGAGCGTCTAATG
GCCTTGAGTCATGAGAATGAGAAATTGAAGGAAGAGCTTGGAAAACTAAA
AGGGAAATCAGAAAGGTCATCTGAGGACCCCACTGATGACTCCAGGCTTC
CCAGGGCCGAAGCGGAGCAGGAAAAGGACCAGCTCAGGACCCAGGTGGTG
AGGCTACAAGCAGAGAAGGCAGACCTGTTGGGCATCGTGTCTGAACTGCA
GCTCAAGCTGAACTCCAGCGGCTCCTCAGAAGATTCCTTTGTTGAAATTA
GGATGGCTGAAGGAGAAGCAGAAGGGTCAGTAAAAGAAATCAAGCATAGT
CCTGGGCCCACGAGAACAGTCTCCACTGGCACGGCATTGTCTAAATATAG
GAGCAGATCTGCAGATGGGGCCAAGAATTACTTCGAACATGAGGAGTTAA
CTGTGAGCCAGCTCCTGCTGTGCCTAAGGGAAGGGAATCAGAAGGTGGAG
AGACTTGAAGTTGCACTCAAGGAGGCCAAAGAAAGAGTTTCAGATTTTGA
AAAGAAAACAAGTAATCGTTCTGAGATTGAAACCCAGACAGAGGGGAGCA
CAGAGAAAGAGAATGATGAAGAGAAAGGCCCGGAGACTGTTGGAAGCGAA
GTGGAAGCACTGAACCTCCAGGTGACATCTCTGTTTAAGGAGCTTCAAGA
GGCTCATACAAAACTCAGCGAAGCTGAGCTAATGAAGAAGAGACTTCAAG
AAAAGTGTCAGGCCCTTGAAAGGAAAAATTCTGCAATTCCATCAGAGTTG
AATGAAAAGCAAGAGCTTGTTTATACTAACAAAAAGTTAGAGCTACAAGT
GGAAAGCATGCTATCAGAAATCAAAATGGAACAGGCTAAAACAGAGGATG
AAAAGTCCAAATTAACTGTGCTACAGATGACACACAACAAGCTTCTTCAA
GAACATAATAATGCATTGAAAACAATTGAGGAACTAACAAGAAAAGAGTC
AGAAAAAGTGGACAGGGCAGTGCTGAAGGAACTGAGTGAAAAACTGGAAC
TGGCAGAGAAGGCTCTGGCTTCCAAACAGCTGCAAATGGATGAAATGAAG
CAAACCATTGCCAAGCAGGAAGAGGACCTGGAAACCATGACCATCCTCAG
GGCTCAGATGGAAGTTTACTGTTCTGATTTTCATGCTGAAAGAGCAGCGA
GAGAGAAAATTCATGAGGAAAAGGAGCAACTGGCATTGCAGCTGGCAGTT
CTGCTGAAAGAGAATGATGCTTTCGAAGACGGAGGCAGGCAGTCCTTGAT
GGAGATGCAGAGTCGTCATGGGGCGAGAACAAGTGACTCTGACCAGCAGG
CTTACCTTGTTCAAAGAGGAGCTGAGGACAGGGACTGGCGGCAACAGCGG
AATATTCCGATTCATTCCTGCCCCAAGTGTGGAGAGGTTCTGCCTGACAT
AGACACGTTACAGATTCACGTGATGGATTGCATCATTtaagcggccgc

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