Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of Optineurin E50K D474N E478A [1 - 577]

Enzyme description:- Optineurin E50K D474N E478A [1 – 577]

Clone number:- DU 4826

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 92, 613.86 daltons
Average Mass 92, 672.22 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.32

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 E478A [1 – 577]

Protein Optineurin E50K D474N E478A [1 – 577]

Clone number DU 4826

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
EDLETMTILRAQMEVYCSNFHAARAAREKIHEEKEQLALQLAVLLKEND
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, a D474N mutation and an E478A mutation.
Residue E50 is equivalent to residue K281 of the fusion protein.
Residue D474 is equivalent to residue N705 of the fusion protein.
And residue E478 is equivalent to residue A709 of the fusion protein.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

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

Complete Nucleotide Sequence:

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCAC
TCGACTTCTTTTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTATG
AGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGTTTG
GAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAACACA
GTCTATGGCCATCATACGTTATATAGCTGACAAGCACAACATGTTGGGTG
GTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTG
GATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACTTTGAAAC
TCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTCG
AAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCATGTAACCCAT
CCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATACATGGACCC
AATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAAAAACGTATTG
AAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGCAAGTATATAGCA
TGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCC
AAAATCGGATCTGGAAGTTCTGTTCCAGGGGCCCCTGGGATCCATGTCCC
ATCAACCTCTCAGCTGCCTCACTGAAAAGGAGGACAGCCCCAGTGAAAGC
ACAGGAAATGGACCCCCCCACCTGGCCCACCCAAACCTGGACACGTTTAC
CCCGGAGGAGCTGCTGCAGCAGATGAAAGAGCTCCTGACCAAGAACCACC
AGCTGAAAGAAGCCATGAAGCTAAATAATCAAGCCATGAAAGGGAGATTT
GAGGAGCTTTCGGCCTGGACAGAGAAACAGAAGGAAGAACGCCAGTTTTT
TGAGATACAGAGCAAAGAAGCAAAAGAGCGTCTAATGGCCTTGAGTCATG
AGAATGAGAAATTGAAGGAAGAGCTTGGAAAACTAAAAGGGAAATCAGAA
AGGTCATCTGAGGACCCCACTGATGACTCCAGGCTTCCCAGGGCCGAAGC
GGAGCAGGAAAAGGACCAGCTCAGGACCCAGGTGGTGAGGCTACAAGCAG
AGAAGGCAGACCTGTTGGGCATCGTGTCTGAACTGCAGCTCAAGCTGAAC
TCCAGCGGCTCCTCAGAAGATTCCTTTGTTGAAATTAGGATGGCTGAAGG
AGAAGCAGAAGGGTCAGTAAAAGAAATCAAGCATAGTCCTGGGCCCACGA
GAACAGTCTCCACTGGCACGGCATTGTCTAAATATAGGAGCAGATCTGCA
GATGGGGCCAAGAATTACTTCGAACATGAGGAGTTAACTGTGAGCCAGCT
CCTGCTGTGCCTAAGGGAAGGGAATCAGAAGGTGGAGAGACTTGAAGTTG
CACTCAAGGAGGCCAAAGAAAGAGTTTCAGATTTTGAAAAGAAAACAAGT
AATCGTTCTGAGATTGAAACCCAGACAGAGGGGAGCACAGAGAAAGAGAA
TGATGAAGAGAAAGGCCCGGAGACTGTTGGAAGCGAAGTGGAAGCACTGA
ACCTCCAGGTGACATCTCTGTTTAAGGAGCTTCAAGAGGCTCATACAAAA
CTCAGCGAAGCTGAGCTAATGAAGAAGAGACTTCAAGATAAGTGTCAGGC
CCTTGAAAGGAAAAATTCTGCAATTCCATCAGAGTTGAATGAAAAGCAAG
AGCTTGTTTATACTAACAAAAAGTTAGAGCTACAAGTGGAAAGCATGCTA
TCAGAAATCAAAATGGAACAGGCTAAAACAGAGGATGAAAAGTCCAAATT
AACTGTGCTACAGATGACACACAACAAGCTTCTTCAAGAACATAATAATG
CATTGAAAACAATTGAGGAACTAACAAGAAAAGAGTCAGAAAAAGTGGAC
AGGGCAGTGCTGAAGGAACTGAGTGAAAAACTGGAACTGGCAGAGAAGGC
TCTGGCTTCCAAACAGCTGCAAATGGATGAAATGAAGCAAACCATTGCCA
AGCAGGAAGAGGACCTGGAAACCATGACCATCCTCAGGGCTCAGATGGAA
GTTTACTGTTCTAATTTTCATGCTGCAAGAGCAGCGAGAGAGAAAATTCA
TGAGGAAAAGGAGCAACTGGCATTGCAGCTGGCAGTTCTGCTGAAAGAGA
ATGATGCTTTCGAAGACGGAGGCAGGCAGTCCTTGATGGAGATGCAGAGT

CGTCATGGGGCGAGAACAAGTGACTCTGACCAGCAGGCTTACCTTGTTCA
AAGAGGAGCTGAGGACAGGGACTGGCGGCAACAGCGGAATATTCCGATTC
ATTCCTGCCCCAAGTGTGGAGAGGTTCTGCCTGACATAGACACGTTACAG
ATTCACGTGATGGATTGCATCATTtaagcggccgc