*Division of Signal Tranduction Therapy*

# Standard Operating Procedure

# Preparation of Optineurin D474N [1 - 577]

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

**Clone number:-** DU 8758

**Source:-** Recombinant

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

**Tag:-** N-terminal GST

**Purification method**:- GSH Sepharose

**Calculated molecular mass:-**
Monoisotopic 92, 686.83 daltons
Average Mass 92, 745.23 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

*Division of Signal Tranduction Therapy*

# Clone Data Sheet

## Optineurin D474N [1 – 577]

**Protein** Optineurin E50K D474N [1 – 577]

**Clone number** DU 8758

**Species** Human

**Accession number** AF420371

**Tags** N-terminal GST

**Bacterially expressed protein**

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA
VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MSHQPLSCLTEKED**
**SPSESTGNGPPHLAHPNLDTFTPEELLQQMKELLTENHQLKEAMKLNNQ**
**AMKGRFEELSAWTEKQKEERQFFEIQSKEAKERLMALSHENEKLKEELG**
**KLKGKSERSSEDPTDDSRLPRAEAEQEKDQLRTQVVRLQAEKADLLGIV**
**SELQLKLNSSGSSEDSFVEIRMAEGEAEGSVKEIKHSPGPTRTVSTGTA**
**LSKYRSRSADGAKNYFEHEELTVSQLLLCLREGNQKVERLEVALKEAKE**
**RVSDFEKKTSNRSEIETQTEGSTEKENDEEKGPETVGSEVEALNLQVTS**
**LFKELQEAHTKLSEAELMKKRLQEKCQALERKNSAIPSELNEKQELVYT**
**NKKLELQVESMLSEIKMEQAKTEDEKSKLTVLQMTHNKLLQEHNNALKT**
**IEELTRKESEKVDRAVLKELSEKLELAEKALASKQLQMDEMKQTIAKQE**
**EDLETMTILRAQMEVYCSNFHAERAAREKIHEEKEQLALQLAVLLKEND**
**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 a D474N mutation. Residue D474 is equivalent to residue N705 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
ACCGAGAACCACCAGCTGAAAGAAGCCATGAAGCTAAATAATCAAGCCAT
GAAAGGGAGATTTGAGGAGCTTTCGGCCTGGACAGAGAAACAGAAGGAAG
AACGCCAGTTTTTTGAGATACAGAGCAAAGAAGCAAAAGAGCGTCTAATG
GCCTTGAGTCATGAGAATGAGAAATTGAAGGAAGAGCTTGGAAAACTAAA
AGGGAAATCAGAAAGGTCATCTGAGGACCCCACTGATGACTCCAGGCTTC
CCAGGGCCGAAGCGGAGCAGGAAAAGGACCAGCTCAGGACCCAGGTGGTG
AGGCTACAAGCAGAGAAGGCAGACCTGTTGGGCATCGTGTCTGAACTGCA
GCTCAAGCTGAACTCCAGCGGCTCCTCAGAAGATTCCTTTGTTGAAATTA
GGATGGCTGAAGGAGAAGCAGAAGGGTCAGTAAAAGAAATCAAGCATAGT
CCTGGGCCCACGAGAACAGTCTCCACTGGCACGGCATTGTCTAAATATAG
GAGCAGATCTGCAGATGGGGCCAAGAATTACTTCGAACATGAGGAGTTAA
CTGTGAGCCAGCTCCTGCTGTGCCTAAGGGAAGGGAATCAGAAGGTGGAG
AGACTTGAAGTTGCACTCAAGGAGGCCAAAGAAAGAGTTTCAGATTTTGA
AAAGAAAACAAGTAATCGTTCTGAGATTGAAACCCAGACAGAGGGGAGCA
CAGAGAAAGAGAATGATGAAGAGAAAGGCCCGGAGACTGTTGGAAGCGAA
GTGGAAGCACTGAACCTCCAGGTGACATCTCTGTTTAAGGAGCTTCAAGA
GGCTCATACAAAACTCAGCGAAGCTGAGCTAATGAAGAAGAGACTTCAAG
AAAAGTGTCAGGCCCTTGAAAGGAAAAATTCTGCAATTCCATCAGAGTTG
AATGAAAAGCAAGAGCTTGTTTATACTAACAAAAAGTTAGAGCTACAAGT
GGAAAGCATGCTATCAGAAATCAAAATGGAACAGGCTAAAACAGAGGATG
AAAAGTCCAAATTAACTGTGCTACAGATGACACACAACAAGCTTCTTCAA
GAACATAATAATGCATTGAAAACAATTGAGGAACTAACAAGAAAAGAGTC
AGAAAAAGTGGACAGGGCAGTGCTGAAGGAACTGAGTGAAAAACTGGAAC
TGGCAGAGAAGGCTCTGGCTTCCAAACAGCTGCAAATGGATGAAATGAAG
CAAACCATTGCCAAGCAGGAAGAGGACCTGGAAACCATGACCATCCTCAG
GGCTCAGATGGAAGTTTACTGTTCTAATTTTCATGCTGAAAGAGCAGCGA
GAGAGAAAATTCATGAGGAAAAGGAGCAACTGGCATTGCAGCTGGCAGTT
CTGCTGAAAGAGAATGATGCTTTCGAAGACGGAGGCAGGCAGTCCTTGAT
GGAGATGCAGAGTCGTCATGGGGCGAGAACAAGTGACTCTGACCAGCAGG
CTTACCTTGTTCAAAGAGGAGCTGAGGACAGGGACTGGCGGCAACAGCGG
AATATTCCGATTCATTCCTGCCCCAAGTGTGGAGAGGTTCTGCCTGACAT
AGACACGTTACAGATTCACGTGATGGATTGCATCATTtaagcggccgc

*Division of Signal Tranduction Therapy*