

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-Optineurin-His

<u>Enzyme description:-</u>	GST-Optineurin-His 1-577
<u>Clone number:-</u>	DU35015
<u>Source:-</u>	BL21 recombinant
<u>Tag:-</u>	N-terminal GST; C-terminal His ₆
<u>Purification method:-</u>	GSH-Sepharose
<u>Expression level:-</u>	1mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	93510 Da
Average Mass	93566 Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	5.37
<u>Purity:-</u>	90%
<u>Enzyme storage buffer:-</u>	
50mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT	
<u>Storage temperature:-</u>	-80°C

Assay:-

Binding assay with poly Ubiquitin (non quantitative)

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Clone Data Sheet

GST-Optineurin-His

<u>Protein</u>	Optineurin 1-577
<u>Synonyms</u>	OPTN, ALS12, FIP2, GLC1E, HIP7, HYPL, NRP, TFIIIA-INTP, FIP-2, HIP-7, E3-14.7K-interacting protein, Huntingtin interacting protein L, huntingtin yeast partner L, huntingtin-interacting protein 7, huntingtin-interacting protein L, nemo-related protein, optic neuropathy-inducing protein, transcription factor IIIA-interacting protein, transcription factor IIIA-interacting protein, tumor necrosis factor alpha-inducible cellular protein containing leucine zipper
<u>Clone Number</u>	DU35015
<u>Species</u>	Human
<u>Accession Number</u>	Protein: Q96CV9
<u>Tags</u>	N-terminal GST; C-terminal His ₆
Amino acid sequence of the expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEF PNLPYYIDGDVKLQSMAIIRYIADKHNMLGGCPKERAIEISMLEGAVLDIRY GVSRIAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDHVTHPDFMLY DALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIAWPLQGWQA TFGGGDHPPKSDLEVLFOGPLGSM SHQPLSCLTEKEDSPSESTGNGPPLAH PNLDTFTPEELLQOMKELLTENHQLKEAMKLNQAMKGRFEELSAWTEKQKE ERQFFEIQSKEAKERLMALSHENEKLKEELGKLGKRSERSSEDPTDDSR LPR AEAEQEKDQLRTOVVRLQAEKADLLGIVSELQKLNSGSSSEDSFVEIRMAE GAEAGSVKEIKHSPGPTRTVSTGTALSKYRSRSADGAKNYFEHEELTVSQLL LCLREGNQKVERLEVALKEAKERVSDFEKKTNRSEIETQTEGSTKENDEE KGPETVGSEVEALNLQVTSLKFELQEAHTKLSEAELMKKRLQEKQALERKN SAIPSELNEKQELVYTNKKLELQVESMLSEIKMEQAKTEDEKSKLTVLQMTN NKLLQEHNNALKTIEELTRKESEKVDRAVLKELSEKLELAEKALASKQLQMD EMKQTI AKQ EEDLETMTILRAQMEVYCSDFHAERAAREKIHEEKEQLALQLA VLLKENDAFEDGGRQSLMEMQSRHGARTSDSDQQAYLVORGAEDRDWRQORN IP IHSCP KCGEVL PDIDTLQIHVMDCI IHHHHHH
Native sequence	in bold
Protease cleavage	Prescission protease site underlined
Cloning sites	BamHI / NotI
<u>DNA sequence of the insert</u>	GGATCCATGTCCCATCAACCTCTCAGCTGCCTCACTGAAAAGGAGGACAG CCCCAGTGAAAGCACAGGAAATGGACCCCCCACCTGGCCCACCCAAACC TGGACACGTTTACCCCGGAGGAGCTGCTGCAGCAGATGAAAGAGCTCCTG ACCGAGAACCACCAGCTGAAAAGAAGCCATGAAGCTAAATAATCAAGCCAT GAAAGGGAGATTTGAGGAGCTTTCGGCCTGGACAGAGAAACAGAAGGAAG AACGCCAGTTTTTTGAGATACAGAGCAAAGAAGCAAAGAGCGTCTAATG

GCCTTGAGTCATGAGAATGAGAAATTGAAGGAAGAGCTTGAAAACTAAA
AGGGAAATCAGAAAGGTCATCTGAGGACCCACTGATGACTCCAGGCTTC
CCAGGGCCGAAGCGGAGCAGGAAAAGGACCAGCTCAGGACCCAGGTGGTG
AGGCTACAAGCAGAGAAGGCAGACCTGTTGGGCATCGTGTCTGAACTGCA
GCTCAAGCTGAACTCCAGCGGCTCCTCAGAAGATTCCTTTGTTGAAATTA
GGATGGCTGAAGGAGAAGCAGAAGGGTCAGTAAAAGAAATCAAGCATAGT
CCTGGGCCCACGAGAACAGTCTCCACTGGCACGGCATTGTCTAAATATAG
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CAAACCATGCCAAGCAGGAAGAGGACCTGGAAACCATGACCATCCTCAG
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GAGAGAAAATTCATGAGGAAAAGGAGCAACTGGCATTGCAGCTGGCAGTT
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AGACACGTTACAGATTCACGTGATGGATTGCATCATTTCATCATCACCATC
ACCATTGAGCGGCCGC