

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of Dynamin-like [1 - 710]

Enzyme description:- Dynamin-like [1 – 710]

Clone number:- DU 30854

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 106, 198.55 daltons

Average Mass 106, 265.68 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.23

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

Dynamin-like [1 - 710]

Protein Dynamin-like [1 – 710]

Clone number DU 30854

Species Human

Accession number NM_012063.2

Tags N-terminal GST

**Bacterially
expressed protein**

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDLFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPCLDAFPKLVCFKKRIEAIPOIDKY
LKSSKYIAWPLOGWQATFGGGDHPKSDLEVLFOGPLGSMEALIPVIN
KLQDVFNTVGADIIQLPQIVVVGTSQSSGKSSVLESVGRDLLPRGTGI
VTRRPLILQLVHVSQEDKRKTTGEENGVEAEWGWKFLHTKNKLYTDFD
EIRQEIENETERISGNKGVSPPEIHLKIFSPNVNLTLDLPGMTKV
PVGDQPKDIELQIRELILRFISNPNSIILAVTAANTDMATSEALKISR
EVDPDGRRTLAVITKLDLMDAGTDAMDVLMGRVIPVKLGIIGVNRSQ
LDINNKKSVTDSIRDEYAFLOKKYPSLANRNGTKYLARTLNRLMHHI
RDCLPELKTRINVLAAQYQSLLSYGEVDDKSATLLQLITKFATEYC
NTIEGTAKYIETSELGGARICYIFHETFGRTLESVDPLGGLNTIDIL
TAIRNATGPRPALFVPEVSFELLVKRQIKRLEEPSLRCVELVHEEMQR
IIQHCSNYSTQELLRFPKLHDAIVEVVTCLLRKRLPVTNEMVHNLVAI
ELAYINTKHPDFADACGLMNNNIEEQRRNRLARELPSAVSRDKLIQDS
RRETKNVASGGGGVGDGVQEPPTGNWRGMLKTSKAEELLAEKSKPIIP
IMPASPQKGHAVNLLDVPVPVARKLSAREQRDCEVIERLIKSYFLIVR
KNIQDSVPKAVMHFLVNHVKDTLQSELVGLYKSSLLDLDLLESEDMA
QRRKEAADMLKALQASQIAEIRETHLW

Native sequence Amino acids M1 – W710 (end) of human Dynamin-like.
Residue M232 of fusion protein is equivalent to M1 of the native
enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage Precission site (LEVLFOGP) at residues 221 – 228

Cloning sites *Bam*H1 and *Not*1 sites of pGex

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Nucleotide
sequence of insert

ggatccATGGAGGCGCTAATTCCTGTCATAAAACAAGCTCCAGGACGTC
TTCAACACGGTGGGCGCCGACATCATCCAGCTGCCTCAAATCGTCGTA
GTGGGAACGCAGAGCAGCGGAAAGAGCTCAGTGCTAGAAAGCCTGGTG
GGGAGGGACCTGCTTCCCAGAGGTACTGGAATTGTCACCCGGAGACCT
CTCATTCTGCAACTGGTCCATGTTTCACAAGAAGATAAACGGAAAACA
ACAGGAGAAGAAAATGGGGTGGAAAGCAGAAGAATGGGGTAAATTTCTT
CACACCAAAAATAAGCTTTACACGGATTTTGATGAAATTCGACAAGAA
ATTGAAAATGAAACAGAAAGAATTTTCAGGAAATAATAAGGGAGTAAGC
CCTGAACCAATTCATCTTAAGATTTTTTCACCCAACGTTGTCAATTTG
ACACTTGTGGATTTGCCAGGAATGACCAAGGTGCCTGTAGGTGATCAA
CCTAAGGATATTGAGCTTCAAATCAGAGAGCTCATTCTTCGGTTCATC
AGTAATCCTAATTCATTATCCTCGCTGTCACTGCTGCTAATACAGAT
ATGGCAACATCAGAGGCACTTAAAATTTCAAGAGAGGTAGATCCAGAT
GGTCGCAGAACCCTAGCTGTAATCACTAACTTGATCTCATGGATGCG
GGTACTGATGCCATGGATGTATTGATGGGAAGGGTTATTCCAGTCAAA
CTTGGAATAATTGGAGTAGTTAACAGGAGCCAGCTAGATATTAACAAC
AAGAAGAGTGTAAC TGATTCAATCCGTGATGAGTATGCTTTTCTTCAA
AAGAAATATCCATCTCTGGCCAATAGAAATGGAACAAAGTATCTTGCT
AGGACTCTAAACAGGTTACTGATGCATCACATCAGAGATTGTTTACCA
GAGTTGAAAACAAGAATAAATGTTCTAGCTGCTCAGTATCAGTCTCTT
CTAAATAGCTACGGTGAACCCGTGGATGATAAAAGTGCTACTTTACTC
CAACTTATTACCAAATTTGCCACAGAATATTGTAACACTATTGAAGGA
ACTGCAAAATATAATTGAACTTCGGAGCTATGCGGTGGTGCTAGAATT
TGTTATATTTTCCATGAGACTTTTGGGCGAACCTTAGAATCTGTTGAT
CCACTTGGTGGCCTTAACACTATTGACATTTTGACTGCCATTAGAAAT
GCTACTGGTCCCTCGTCCCTGCTTTATTTGTGCCTGAGGTTTCATTTGAG
TTACTGGTGAAGCGGCAAATCAAACGCTTAGAAGAGCCCAGCCTCCGC
TGTGTGGAAC TGGTTCATGAGGAAATGCAAAGGATCATTCAGCACTGT
AGCAATTACAGTACACAGGAATTGTTACGATTTCCATAA ACTTCATGAT
GCCATAGTTGAAGTGGT GACTTGTCTTCTTCGTA AAAGGTTGCC TGT
ACAAATGAAATGGTCCATAACTTAGTGGCAATTGAACTGGCTTATATC
AACACAAAACATCCAGACTTTGCTGATGCTTGTGGGCTAATGAACAAT
AATATAGAGGAACAAAGGAGAAACAGGCTAGCCAGAGAATTACCTTCA
GCTGTATCACGAGACAAGTTAATTCAGGACAGCAGAAGAGAAACTAAA
AATGTTGCATCTGGAGGTGGTGGGGTTGGAGATGGTGTTC AAGAACCA
ACCACAGGCAACTGGAGAGGAATGCTGAAA ACTTCAAAGCTGAAGAG
TTATTAGCAGAAGAAAAATCAAACCCATTC CAATTATGCCAGCCAGT
CCACAAAAGGTCATGCCGTGAACCTGCTAGATGTGCCAGTTCCCTGTT
GCACGAAA ACTATCTGCTCGGGAACAGCGAGATTGTGAGGTTATTGAA
CGACTCATTAATCATATTTTCTCATTGTCAGAAAGAATATTCAAGAC
AGTGTGCCAAAGGCAGTAATGCATTTTTTTGGTTAATCATGTGAAAGAC
ACTCTT CAGAGTGAGCTAGTAGGCCAGCTGTATAAATCATCCTTATTG
GATGATCTTCTGACAGAACTGAGGACATGGCACAGCGCAGGAAAGAA
GCAGCTGATATGCTAAAGGCATTACAAGGAGCCAGTCAAAT TATTGCT
GAAATCCGGGAGACTCATCTTTGGtgagcggccgc