Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of MIRO1 [1 – 592]

Enzyme description:- MIRO1 [1 – 592]

Clone number:- DU 40832

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal His6 tag

Purification method:- Cobalt resin

Calculated molecular mass:
Monoisotopic 79, 800.14 daltons
Average Mass 79, 851.08 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.71

Purity:- 85 %

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, 0.2 mM PMSF, 1 mM Benzamidine

Storage temperature:- –70 °C

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Clone Data Sheet

MIRO1 [1 – 592]

Protein	MIRO1 [1 - 592]
Clone number	DU 40832
Species	Human
Accession number	NM_018307.3 with silent changes g18a and c21t
Tags	N-terminal His6
Bacterially expressed protein	MGHHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKV KMTTHLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGME EEDVIEVYQEQTGG**MKKDVRILLVGEPRVGKTSLIMSLVSEEFPE EVPPRAEEITIPADVTPERVPTHIVDYSEAEQSDEQLHQEISQAN VICIVYAVNNKHSIDKVTSRWIPLINERTDKDSRLPLILVGNKSD LVEYSSMETILPIMNQYTEIETCVECSAKNLKNISELFYYAQKAV LHPTGPLYCPEEKEMKPACIKALTRIFKISDQDNDGTLNDAELNF FQRICFNTPLAPQALEDVKNVVRKHISDGVADSGLTLKGFLFLHT LFIQRGRHETTWTVLRRFGYDDDLDLTPEYLFPLLKIPPDCTTEL NHHAYLFLQSTFDKHDLDRDCALSPDELKDLFKVFPYIPWGPDVN NTVCTNERGWITYQGFLSQWTLTTYLDVQRCLEYLGYLGYSILTE QESQASAVTVTRDKKIDLQKKQTQRNVFRCNVIGVKNCGKSGVLQ ALLGRNLMRQKKIREDHKSYYAINTVYVYGQEKYLLLHDISESEF LTEAEIICDVVCLVYDVSNPKSFEYCARIFKQHFMDSRIPCLIVA AKSDLHEVKQEYSISPTDFCRKHKMPPPQAFTCNTADAPSKDIFV KLTTMAMYPHVTQADLKSSTF**
Native sequence	Amino acids M1 – F592 (end residue is R618) of human MIRO1. Residue M105 of the fusion protein is equivalent to M1 of the native enzyme. The His6 tag is located at residues 3 - 8.
Protease cleavage	SENP1 cleavge of SUMO: (SDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTT HLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGME EEDVIEVYQEQTGG) residues 9 - 104
Vector	pET6His

Nucleotide sequence

ATGGGTCATCATCACCATCACCATTCTGACCAGGAGGCAAAACCT
TCAACTGAGGACTTGGGGGATAAGAAGGAAGGTGAATATATTAAA
CTCAAAGTCATTGGACAGGATAGCAGTGAGATTCACTTCAAAGTG
AAAATGACAACACATCTCAAGAAACTCAAAGAATCATACTGTCAA
AGACAGGGTGTTCCAATGAACTCACTCAGGTTTCTCTTTGAGGGT
CAGAGAATTGCTGATAATCATACTCCAAAAGAACTGGGAATGGAG
GAAGAAGATGTGATTGAAGTTTATCAGGAACAAACGGGGGGAATG
AAGAAAGACGTGCGAATTCTGCTGGTGGGAGAACCTAGAGTTGGG
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GAACAGAGTGATGAACAACTTCATCAAGAAATATCTCAGGCTAAT
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TGCCTGGAATATTTGGGCTATCTAGGCTATTCAATATTGACTGAG
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CCTACTGATTTCTGCAGGAAACACAAAATGCCTCCACCACAAGCC
TTCACTTGCAATACTGCTGATGCCCCCAGTAAGGATATCTTTGTT
AAATTGACAACAATGGCCATGTATCCGCACGTGACACAAGCTGAC
CTCAAGAGCTCCACGTTTtga

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