

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-Halo-NEMO

<u>Enzyme description:-</u>	His-Halo-NEMO (2-419)
<u>Clone number:-</u>	DU23728
<u>Source:-</u>	human Recombinant
<u>Tag:-</u>	N-terminal His-TEV
<u>Purification method:-</u>	Ni ⁺⁺ -NTA-Sepharose, SEC
<u>Expression system:-</u>	E.coli
<u>Calculated molecular mass:-</u>	
Monoisotopic	83729
Average Mass	83778
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	5.54
<u>Purity:-</u>	90%
<u>Enzyme storage buffer:-</u>	
50 mM HEPES pH 7.5, 150mM NaCl, 1mM DTT	
<u>Storage temperature:-</u>	-80°C
<u>Assay:-</u>	Immobilise covalently onto Chloralkane resin and use for the capture of Met-linked and K63 linked poly ubiquitin chains.

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Clone Data Sheet

Protein name His-Halo-NEMO

<u>Protein</u>	His-Halo-NEMO 2-419
<u>Synonyms</u>	IKK-gamma
<u>Clone Number</u>	DU23728
<u>Species</u>	human
<u>Accession Number</u>	Protein: Q9Y6K9
<u>Tags</u>	N-terminal His-TEV-
Aminoacid sequence of the expressed protein	MGSSHHHHHHENLYFQMAEIGTGFPFDPHYVEVLGERMHYVDVGPRD GTPVLFLLHGNPTSSYVWRNIIPHVAPTHRCIAPDLIGMGKSDKPDLG YFFDDHVRFMDAFIEALGLEEVVLLVIHDWGSALGFHWAKRNPERSVKG IAFMFIRPIPTWDEWPEFARETFQAFRTTDVGRKLIIDQNVFIEGT LPMGVVRPLTEVEMDHYREPFLNPVDREPLWRFPNELPIAGEPANIV ALVEEYMDWLHQSPVPKLLFWGTPGVLIPPAEAARLAKSLPNCKAVD IGPGLNLLQEDNPDLLIGSEIARWLSTLEISGGSNRHLWKSQLCENVQ PSGGPAADQDVLGGEESPLGKPAMLHLPSEQGAPETLQRCLEENQELR DAIRQSNQILRERCEELLHFQASQREEKEFLMCKFQEARLVERLGL EKLDLKRQEQALREVEHLKRCQQQMAEDKASVKAQVTSLLGELQES QSRLEAATKECQALEGRARAASEQARQLESEREALQQQHSVQVDQLR MQGQSVEAALRMERQAASEEKRKLAQLQVAYHQLFQEYDNHIKSSVV GSEKRGMQLEDLKQQLQQAEEALVAKQEVIDKLKEEAEQHKIVMET VPVLKAQADIYKADFQAEQAREKLAEKKELLQEQLEQLOREYSKLG ASCQESARIEDMRKRHVEVSQAPLPPAPAYLSSPLALPSQRRSPPEE PPDFCCPKCQYQAPDMDTLQIHVMECIE
Native sequence	in bold NEMO residues 2 - 419, representing full length with the start Met. Halo-tag in italic
Protease cleavage	TEV protease underlined
Cloning sites	BamHI / Not1

**DNA sequence
of the expression
cassette**

ATGGGCAGCAGCCATCATCATCATCACGAAAACCTGTATTTTCAGatggc
agaaatcggtagtggctttccattcagacccccattatgtggaagtcctgggcg
agcgcacgactacgctcgatggtgggtccgcgcgatggcaccctgtgctgttc
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tgcaccgaccatcgctgcattgctccagacctgatcggatgggcaaatccg
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AAGCTCGATCTGAAGAGGCAGAAGGAGCAGGCTCTGCGGGAGGTGGAGCACCT
GAAGAGATGCCAGCAGCAGATGGCTGAGGACAAGGCCTCTGTGAAAGCCCAGG
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CGCCAGGCCGCTCGGAGGAGAAGAGGAAGCTGGCCAGTTGCAGGTGGCCTA
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GTGAGCGGAAGCGAGGAATGCAGCTGGAAGATCTCAAACAGCAGCTCCAGCAG
GCCGAGGAGGCCCTGGTGGCCAAACAGGAGGTGATCGATAAGCTGAAGGAGGA
GGCCGAGCAGCACAAGATTGTGATGGAGACCGTTCCGGTGTGAAGGCCCAGG
CGGATATCTACAAGGCGGACTTCCAGGCTGAGAGGCAGGCCCGGGAGAAGCTG
GCCGAGAAGAAGGAGCTCCTGCAGGAGCAGCTGGAGCAGCTGCAGAGGGAGTA
CAGCAAACCTGAAGGCCAGCTGTCAGGAGTCGGCCAGGATCGAGGACATGAGGA
AGCGGCATGTCGAGGTCTCCAGGCCCTTGCCCCCGCCCCCTGCCACCTC
TCCTCTCCCCCTGGCCCTGCCAGCCAGAGGAGGAGCCCCCCCCGAGGAGCCACC
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