

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-NEMO-His

<u>Enzyme description:-</u>	GST-NEMO-His 2-419
<u>Clone number:-</u>	DU35062
<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	75665 Da
Average Mass	75710 Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	5.86
<u>Purity:-</u>	80%
<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-NEMO-His

<u>Protein</u>	NEMO 2-419
<u>Synonyms</u>	AMCBX1, FIP-3, FIP3, Fip3p, IKK-gamma, IP, IP1, IP2, IPD2, IKKAP1, IKKG, I-kappa-B kinase subunit gamma, Ikb kinase gamma subunit, NF-kappa-B essential modifier, NF-kappa-B essential modulator, Nf-kappaB essential modulator, ikB kinase subunit gamma, ikB kinase-associated protein 1, incontinentia pigmenti, inhibitor of nuclear factor kappa-B kinase subunit
<u>Clone Number</u>	DU35062
<u>Species</u>	Human
<u>Accession Number</u>	Protein: Q9Y6K9
<u>Tags</u>	N-terminal GST; C-terminal His ₆
Aminoacid sequence of the expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEHLERDEGDKWRNKKFELGLEF PNLPYYIDGDVKLQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRY GVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDHVTHPDFMLY DALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIAWPLOGWQA TFGGGDHPPKSDLEVLFOGPLGS NRHLWKSQ LC EMVQPSGGPAADQ DVL GEE SPLGKPAMLHLPSEQGAPETLQRCLEENQELRDAIRO SNQILRERCEELLHF QASQREEKEFLMCKFQEAR KLVERLGLEKLDLKR QEQALREVEHLKRCQQQ MAEDKASVKAQVTSLLGELQESQSRLEAATKECQALEGRARAASEQARQLES EREALQQQHSVQVDQLRMQGSVEAALRMERQAASEEK RKLAQLQVAYHQLF QEYDNHIKSSVVGSE RKRG MQLEDLQQLQQAEEALVAKQ EVIDKLKEEA EQ HKIVMETVPVLKAQADIYKADFQ AERQAREKLA EKKELLQEQLEQLOREYSK LKASCQESARIEDMRKRHVEVSQAPLPPAPAYLSSPLALPSQRRSPPEEPPD FCCPKCQYQAPDMDTLQIHVMECIEHHHHHHH
Native sequence	in bold
Protease cleavage	Pre-scission protease site underlined
Cloning sites	BamHI / NotI
<u>DNA sequence of the insert</u>	GGATCCAATAGGCACCTCTGGAAGAGCCA ACTGTGTGAGATGGTGCAGCC CAGTGGTGGCCCGGCAGCAGATCAGGACGTACTGGGCGAAGAGTCTCCTC TGGGGAAGCCAGCCATGCTGCACCTGCCTTCAGAACAGGGCGCTCCTGAG ACCCTCCAGCGCTGCCTGGAGGAGAATCAAGAGCTCCGAGATGCCATCCG GCAGAGCAACCAGATTCTGCGGGAGCGCTGCGAGGAGCTTCTGCATTTCC AAGCCAGCCAGAGGGAGGAGAAGGAGTTCCTCATGTGCAAGTCCAGGAG GCCAGGAACTGGTGGAGAGACTCGGCCTGGAGAAGCTCGATCTGAAGAG GCAGAAGGAGCAGGCTCTGCGGGAGGTGGAGCACCTGAAGAGATGCCAGC AGCAGATGGCTGAGGACAAGGCCCTCTGTGAAAGCCAGGTGACGTCCTTG CTCGGGGAGCTGCAGGAGAGCCAGAGTCGCTTGGAGGCTGCCACTAAGGA ATGCCAGGCTCTGGAGGGTTCGGGCCCGGGCGGCCAGCGAGCAGGCGCGGC

AGCTGGAGAGTGAGCGCGAGGCGCTGCAGCAGCAGCACAGCGTGCAGGTG
GACCAGCTGCGCATGCAGGGCCAGAGCGTGGAGGCCGCGCTCCGCATGGA
GCGCCAGGCCGCTCGGAGGAGAAGAGGAAGCTGGCCCAGTTGCAGGTGG
CCTATCACCAGCTCTTCCAAGAATACGACAACCACATCAAGAGCAGCGTG
GTGGGCAGTGAGCGGAAGCGAGGAATGCAGCTGGAAGATCTCAAACAGCA
GCTCCAGCAGGCCGAGGAGGCCCTGGTGGCCAAACAGGAGGTGATCGATA
AGCTGAAGGAGGAGGCCGAGCAGCACAAAGATTGTGATGGAGACCGTTCCG
GTGCTGAAGGCCAGGCGGATATCTACAAGGCGGACTTCCAGGCTGAGAG
GCAGGCCCGGGAGAAGCTGGCCGAGAAGAAGGAGCTCCTGCAGGAGCAGC
TGGAGCAGCTGCAGAGGGAGTACAGCAAACCTGAAGGCCAGCTGTCAGGAG
TCGGCCAGGATCGAGGACATGAGGAAGCGGCATGTCGAGGTCTCCCAGGC
CCCCTTGCCCCCGCCCCTGCCTACCTCTCCTCTCCCCTGGCCCTGCCCA
GCCAGAGGAGGAGCCCCCGAGGAGCCACCTGACTTCTGCTGTCCAAG
TGCCAGTATCAGGCCCTGATATGGACACCCTGCAGATACATGTCATGGA
GTGCATTGAGCATCATCACCATCACCATTGAGCGGCCGC