

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-UBE2M

<u>Enzyme description:-</u>	His-UBE2M
<u>Clone number:-</u>	DU15804
<u>Source:-</u>	BL21 recombinant
<u>Tag:-</u>	N-terminal His ₆ -tag
<u>Purification method:-</u>	Ni ⁺⁺ -NTA-Sepharose
<u>Expression level:-</u>	5mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	24499 Da
Average Mass	24514 Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	7.24
<u>Purity:-</u>	90%
<u>Enzyme storage buffer:-</u>	
50mM HEPES pH 7.5, 150mM NaCl, 10% glycerol, 1mM DTT	
<u>Storage temperature:-</u>	-80°C
<u>Assay:-</u>	
Loading assay with NEDD8 and NAE1/NAE2 in the presence of Mg-ATP	

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Clone Data Sheet

His-UBE2M

<u>Protein</u>	UBE2M
<u>Synonyms</u>	Ubc12
<u>Clone Number</u>	DU15804
<u>Species</u>	Human
<u>Accession Number</u>	Protein: P61081 DNA: NM_003969
<u>Tags</u>	N-terminal His ₆ tag
Aminoacid sequence of the expressed protein	MGSSHHHHHSSG <u>LEVL</u> FQGPSP EF PGVDSKAAAM IKLFSLKQOKKEE SAGG TKGSSKKASAAQLRIQKDINELNLPKTC DI SFSDPDDLNFKL VIC PDE GFYKSGKFVFSFKVGGYPHDPPKVKCETMVYHPNIDLEGNVCLN IL RED WKPVL TINSI IYGLQYLFLEPNPEDPLNKEAAEVLQNNRRLFEQ NVQ RSM RGYIGSTYFER CLK
Native sequence	in bold
Protease cleavage	Prescission site underlined
Cloning sites	Not1
<u>DNA sequence of insert</u>	GCGGCCGCGATGATCAAGCTGTTCTCGCTGAAGCAGCAGAAGAAGGAGGA GGAGTCGGCGGGCGGCACCAAGGGCAGCAGCAAGAAGGCGTCGGCGGGCGC AGCTGCGGATCCAGAAGGACATAAACGAGCTGAACCTGCCCAAGACGTGT GATATCAGCTTCTCAGATCCAGACGACCTCCTCAACTTCAAGCTGGTCAT CTGTCCTGATGAGGGCTTCTACAAGAGTGGGAAGTTTGTGTTTCAGTTTTA AGGTGGGCCAGGGTTACCCGCATGATCCCCCAAGGTGAAGTGTGAGACA ATGGTCTATCACCCCAACATTGACCTCGAGGGCAACGTCTGCCTCAACAT CCTCAGAGAGGACTGGAAGCCAGTCCTTACGATAAACTCCATAATTTATG GCCTGCAGTATCTTCTTGGAGCCCAACCCCGAGGACCCACTGAACAAG GAGCCGCAGAGGTCTGCAGAACAACCGGGCGCTGTTTTGAGCAGAACGT GCAGCGCTCCATGCGGGGTGGCTACATCGGCTCCACCTACTTTGAGCGCT GCCTGAAATAGGCGGCCGC