

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-UBE2F

<u>Enzyme description:-</u>	His-UBE2F
<u>Clone number:-</u>	DU14052
<u>Source:-</u>	BL21 recombinant
<u>Tag:-</u>	N-terminal His ₆ -tag
<u>Purification method:-</u>	Ni ⁺⁺ -NTA-Sepharose
<u>Expression level:-</u>	15mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	24605 Da
Average Mass	24620 Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	7.7
<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

Assay:-

Loading assay with NEDD8 and NAE1/NAE2 in the presence of Mg-ATP

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Clone Data Sheet

His-UBE2F

<u>Protein</u>	UBE2F
<u>Synonyms</u>	
<u>Clone Number</u>	DU14052
<u>Species</u>	Human
<u>Accession Number</u>	Protein: Q969M7 DNA: NM_080678
<u>Tags</u>	N-terminal His ₆ tag
Aminoacid sequence of the expressed protein	MGSSHHHHHSSGLVPRGSHMASMTGGQOMGRGSMLTLASKLKRDDGLKG SRTAATASDSTRRVSVRDKLLVKEVALEANLPCTCKVHFDPNKLHCFQ LTVTPDEGYIQGGKQFETEVDPAYNMVPPKVKCLTKIWHPNITETGEIC LSLLEHSIDGTGWAPTRTLKDVVWGLNSLFTDLLNFDDPLNIEAAEHL RDKEDFRNKVDDYIKRYAR
Native sequence	in bold
Protease cleavage	Thrombin site underlined
Cloning sites	BamH1 / Not1
<u>DNA sequence of insert</u>	GGATCCATGCTAACGCTAGCAAGTAACTGAAGCGTGACGATGGTCTCAA AGGGTCCCAGGACGGCAGCCACAGCGTCCGACTCGACTCGGAGGGTTTCTG TGAGAGACAAATTGCTTGTTAAAGAGGTTGCAGAACTTGAAGCTAATTTA CCTTGTACATGTAAAGTGCATTTTCTTGATCCAAACAAGCTTCATTGTTT TCAGCTAACAGTAACCCAGATGAGGGTTACTACCAGGGTGGAAAATTTT AGTTTGAACTGAAGTTCCCGATGCGTACAACATGGTGCCTCCCAAAGTG AAATGCCTGACCAAGATCTGGCACCCCAACATCACAGAGACAGGGGAAAT ATGCTGAGTTTATTGAGAGAACATTC AATTGATGGCACTGGCTGGGCTC CCACAAGAACATTAAAGGATGTCGTTTGGGGATTAAACTCTTTGTTTACT GATCTTTTGAATTTGATGATCCACTGAATATTGAAGCTGCAGAACATCA TTTGCGGGACAAGGAGGACTTCCGGAATAAAGTGGATGACTACATCAAAC GTTATGCCAGATGAGCGGCCGC