

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-UBE2H

<u>Enzyme description:-</u>	His-UBE2H
<u>Clone number:-</u>	DU32149
<u>Source:-</u>	BL21 recombinant
<u>Tag:-</u>	N-terminal His ₆ -tag
<u>Purification method:-</u>	Ni ⁺⁺ -NTA-Sepharose
<u>Expression level:-</u>	7mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	23085 Da
Average Mass	23099 Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	4.84
<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 with Ubiquitin and UBE1 in the presence of Mg-ATP	

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Clone Data Sheet

His-UBE2H

<u>Protein</u>	UBE2H
<u>Synonyms</u>	Ubc8, E2-20K, UbcH, UbcH2
<u>Clone Number</u>	DU32149
<u>Species</u>	Human
<u>Accession Number</u>	Protein: NP_003335, DNA: NM_003344
<u>Tags</u>	N-terminal His ₆ tag
Aminoacid sequence of the expressed protein	MGSSHHHHHSSGLEVLFGPGSMSSPSPGKRRMDTDVVKLIESKHEVTI LGGLNEFVVKFYGPQGTPIEGGVWVRVDLPDKYPFKSPSIGFMNKIFHP NIDEASGTVCLDVINQWTALYDLTNIFESFLPQLLAYPNPIDPLNGDAA AMYLHRPEEYKQKIKEYIQKYATEEALKEQEETGDSSSESSMSDFSEDE AQDMEL
Native sequence	in bold
Protease cleavage	Prescission Protease site underlined
Cloning sites	BamH1 / NotI
<u>DNA sequence of insert</u>	GGATCCATGTCATCTCCCAGTCCGGGCAAGAGGCGGATGGACACGGACGT GGTCAAGCTCATCGAGAGTAAACATGAGGTTACGATCCTGGGAGGACTTA ATGAATTTGTAGTGAAGTTTTATGGACCACAAGGAACACCATATGAAGGC GGAGTATGGAAAGTTAGAGTGGACCTACCTGATAAATACCCTTTCAAATC TCCATCTATAGGATTCATGAATAAAATTTTCCATCCCAACATTGATGAAG CGTCAGGAAGTGTGTGTCTAGATGTAATTAATCAAACCTTGGACAGCTCTC TATGATCTTACCAATATATTTGAGTCCTTCCTGCCTCAGTTATTGGCCTA TCCTAACCCCATAGATCCTCTCAATGGTGACGCTGCAGCCATGTACCTCC ACCGACCAGAAGAATACAAGCAGAAAATTAAAGAGTACATCCAGAAATAC GCCACGGAGGAGGCGCTGAAAGAACAGGAAGAGGGTACCGGGGACAGCTC ATCGGAGAGCTCTATGTCTGACTTTTCCGAAGATGAGGCCAGGATATGG AGTTGTAGGCGGCCGC