Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of UBE2H

Enzyme description:- UBE2H

Clone number:- DU32149

Source:- BL21 recombinant

Tag:- cleaved from N-terminal His_6-tag

Purification method:- Ni^{++}-NTA-Sepharose, protease treatment

Expression level:- 2.5mg/L

Calculated molecular mass:-

Monoisotopic	20940 Da
Average Mass	20952 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 4.47

Purity:- 90%

Enzyme storage buffer:-

50mM HEPES pH 7.5, 150mM NaCl, 10% glycerol, 1mM DTT

Storage temperature:- -80ºC

Assay:-

Loading with Ubiquitin and UBE1 in the presence of Mg-ATP

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Clone Data Sheet

His-UBE2H

Protein	UBE2H
Synonyms	Ubc8, E2-20K, UbcH, UbcH2
Clone Number	DU32149
Species	Human
Accession Number	Protein: NP_003335, DNA: NM_003344
Tags	N-terminal His_6 tag
Aminoacid sequence of the expressed protein	GPGS**MSSPSPGKRRMDTDVVKLIESKHEVTILGGLNEFVVKFYGPQGTPYEG GVWKVRVDLPDKYPFKSPSIGFMNKIFHPNIDEASGTVCLDVINQTWTALYD LTNIFESFLPQLLAYPNPIDPLNGDAAAMYLHRPEEYKQKIKEYIQKYATEE ALKEQEEGTGDSSSESSMSDFSEDEAQDMEL**
Native sequence	in bold
Protease cleavage	Prescission Protease site underlined
Cloning sites	BamH1 / Not1

DNA sequence of insert

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GGATCCATGTCATCTCCCAGTCCGGGCAAGAGGCGGATGGACACGGACGT
GGTCAAGCTCATCGAGAGTAAACATGAGGTTACGATCCTGGGAGGACTTA
ATGAATTTGTAGTGAAGTTTTATGGACCACAAGGAACACCATATGAAGGC
GGAGTATGGAAAGTTAGAGTGGACCTACCTGATAAATACCCTTTCAAATC
TCCATCTATAGGATTCATGAATAAAATTTTCCATCCCAACATTGATGAAG
CGTCAGGAACTGTGTGTCTAGATGTAATTAATCAAACTTGGACAGCTCTC
TATGATCTTACCAATATATTTGAGTCCTTCCTGCCTCAGTTATTGGCCTA
TCCTAACCCCATAGATCCTCTCAATGGTGACGCTGCAGCCATGTACCTCC
ACCGACCAGAAGAATACAAGCAGAAAATTAAAGAGTACATCCAGAAATAC
GCCACGGAGGAGGCGCTGAAAGAACAGGAAGAGGGTACCGGGGACAGCTC
ATCGGAGAGCTCTATGTCTGACTTTTCCGAAGATGAGGCCCAGGATATGG
AGTTGTAGGCGGCCGC
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