Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of UBE2B

Enzyme description:- UBE2B

Clone number:- DU20017

Source:- BL21 recombinant

Tag:- cleaved from N-terminal His_6-tag

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

Expression level:- 6mg/L

Calculated molecular mass:-

Monoisotopic	17599 Da
Average Mass	17610 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 4.81

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

Division of Signal Transduction Therapy

Clone Data Sheet

His-UBE2B

Protein	UBE2B
Synonyms	ubiquitin-conjugating enzyme E2B, Rad 6 homolog
Clone Number	DU20017
Species	Human
Accession Number	Protein: NP_003328 DNA: NM_003337
Tags	N-terminal His_6 tag, cleaved
Aminoacid sequence of the expressed protein	GPGS**MSTPARRRLMRDFKRLQEDPPVGVSGAPSENNIMQWNAVIFGPEGTPF EDGTFKLVIEFSEEYPNKPPTVRFLSKMFHPNVYADGSICLDILQNRWSPTY DVSSILTSIQSLLDEPNPNSPANSQAAQLYQENKREYEKRVSAIVEQSWNDS**
Native sequence	
Protease cleavage	Prescission site underlined
Cloning sites	BamH1 / Not1

DNA sequence of insert

GGATCCATGTCGACCCCGGCCCGGAGGAGGCTCATGCGGGATTTCAAGCG
GTTACAAGAGGACCCACCTGTGGGTGTCAGTGGCGCACCATCTGAAAACA
ACATCATGCAGTGGAATGCAGTTATATTTGGACCAGAAGGGACACCTTTT
GAAGATGGTACTTTTAAACTAGTAATAGAATTTTCTGAAGAATATCCAAA
TAAACCACCAACTGTTAGGTTTTTATCCAAAATGTTTCATCCAAATGTGT
ATGCTGATGGTAGCATATGTTTAGATATCCTTCAGAATCGATGGAGTCCA
ACATATGATGTATCTTCTATCTTAACATCAATTCAGTCTCTGCTGGATGA
ACCGAATCCTAACAGTCCAGCCAATAGCCAGGCAGCACAGCTTTATCAGG
AAAACAAACGAGAATATGAGAAAAGAGTTTCGGCCATTGTTGAACAAAGC
TGGAATGATTCATAAGCGGCCGC