

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-UBE2B

Enzyme description:- His-UBE2B 1-152 (full length)

Clone number:- DU20017

Source:- human recombinant

Tag:- N-terminal His₆-tag

Purification method:- Ni⁺⁺-NTA-Sepharose

Expression system:- E.coli

Calculated molecular mass:-

Monoisotopic 19744 Da

Average Mass 19756 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.61

Purity:- 90%

Enzyme storage buffer:-

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

Storage temperature:- -80°C

Assay:-

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

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Clone Data Sheet

His-UBE2B

<u>Protein</u>	His-UBE2B 1-152 (full length)
<u>Synonyms</u>	ubiquitin-conjugating enzyme E2B, Rad 6 homolog
<u>Clone Number</u>	DU20017
<u>Species</u>	Human
<u>Accession Number</u>	Protein: NP_003328 DNA: NM_003337
<u>Tags</u>	N-terminal His ₆ tag
Aminoacid sequence of the expressed protein	MGSSHHHHHSSG <u>LEVL</u> FQGP SMSTPARRRLMRDFKRLQEDPPVGVSGA PSENNIMQWNAVIFGPEGTFEDGTFKLVIEFSEEYPNKPPTVRFLSKMF HPNVYADGSICLDILQNRWSPTYDVSSILTSIQSLLDEPNPNSPANSQAA QLYQENKREYEKRVSAIVEQSWNDS
Native sequence	in bold
Protease cleavage	Prescission site underlined
Cloning sites	BamH1 / NotI
<u>DNA sequence of insert</u>	<u>GGATCC</u> ATGTCGACCCCGGCCCGGAGGAGGCTCATGCGGGATTTCAAGCG GTTACAAGAGGACCCACCTGTGGGTGTCAGTGCGCACCATCTGAAAACA ACATCATGCAGTGGAAATGCAGTTATATTTGGACCAGAAGGGACACCTTTT GAAGATGGTACTTTTAAACTAGTAATAGAATTTTCTGAAGAATATCCAAA TAAACCACCAACTGTTAGGTTTTTATCCAAAATGTTTCATCCAAATGTGT ATGCTGATGGTAGCATATGTTTAGATATCCTTCAGAATCGATGGAGTCCA ACATATGATGTATCTTCTATCTTAACATCAATTCAGTCTCTGCTGGATGA ACCGAATCCTAACAGTCCAGCCAATAGCCAGGCAGCACAGCTTTATCAGG AAAACAAACGAGAATATGAGAAAAGAGTTTCGGCCATTGTTGAACAAAGC TGGAATGATTCATAAGCGGCCGC