Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of UBE2L3

Enzyme description:- UBE2L3

Clone number:- DU12798

Source:- BL21 recombinant

Tag:- cleaved from N-terminal His_6-tag

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

Expression level:- 10mg/L

Calculated molecular mass:-

Monoisotopic	18148 Da
Average Mass	18159Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 9.28

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

UBE2L3

Protein	UBE2L3
Synonyms	UbcH7
Clone Number	DU12798
Species	Human
Accession Number	Protein: P68036 DNA: NM_003347
Tags	N-terminal His_6 tag (cleaved)
Aminoacid sequence of the expressed protein	GPGS**MAASRRLMKELEEIRKCGMKNFRNIQVDEANLLTWQGLIVPDNPPYDK GAFRIEINFPAEYPFKPPKITFKTKIYHPNIDEKGQVCLPVISAENWKPATK TDQVIQSLIALVNDPQPEHPLRADLAEEYSKDRKKFCKNAEEFTKKYGEKRP VD**
Native sequence	in bold
Protease cleavage	Prescission site underlined
Cloning sites	BamH1 / Not1

DNA sequence of insert

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GGATCCATGGCGGCCAGCAGGAGGCTGATGAAGGAGCTTGAAGAAATCCG
CAAATGTGGGATGAAAAACTTCCGTAACATCCAGGTTGATGAAGCTAATT
TATTGACTTGGCAAGGGCTTATTGTTCCTGACAACCCTCCATATGATAAG
GGAGCCTTCAGAATCGAAATCAACTTTCCAGCAGAGTACCCATTCAAACC
ACCGAAGATCACATTTAAAACAAAGATCTATCACCCAAACATCGACGAAA
AGGGGCAGGTCTGTCTGCCAGTAATTAGTGCCGAAAACTGGAAGCCAGCA
ACCAAAACCGACCAAGTAATCCAGTCCCTCATAGCACTGGTGAATGACCC
CCAGCCTGAGCACCCGCTTCGGGCTGACCTAGCTGAAGAATACTCTAAGG
ACCGTAAAAAATTCTGTAAGAATGCTGAAGAGTTTACAAAGAAATATGGG
GAAAAGCGACCTGTGGACTAAGCGGCCGC
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