Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-UBE2D3

Enzyme description:- His-UBE2D3 2-147 (end)

Clone number:- DU15703

Source:- human recombinant

Tag:- N-terminal His_6-tag

Purification method:- Ni^{++}-NTA-Sepharose

Expression system:- E.coli

Calculated molecular mass:-

Monoisotopic	18988 Da
Average Mass	19000 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 7.7

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-UBE2D3

Protein	His-UBE2D3 2-147 (end)
Synonyms	UbcH5c, E2D3
Clone Number	DU15703
Species	Human
Accession Number	Protein: P61077 DNA: NM_003340
Tags	N-terminal His_6 tag
Aminoacid sequence of the expressed protein	MGSSHHHHHHSSGLEVLFQGPGS**ALKRINKELSDLARDPPAQCSAGPVGD DMFHWQATIMGPNDSPYQGGVFFLTIHFPTDYPFKPPKVAFTTRIYHPNI NSNGSICLDILRSQWSPALTISKVLLSICSLLCDPNPDDPLVPEIARIYK TDRDKYNRISREWTQKYAM**
Native sequence	Start Methionine is missing
Protease cleavage	Prescission site underlined
Cloning sites	BamH1 / Not1

DNA sequence of insert

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GGATCCGCGCTGAAACGGATTAATAAGGAACTTAGTGATTTGGCCCGTGA
CCCTCCAGCACAATGTTCTGCAGGTCCAGTTGGGGATGATATGTTTCATT
GGCAAGCCACAATTATGGGACCTAATGACAGCCCATATCAAGGCGGTGTA
TTCTTTTTGACAATTCATTTTCCTACAGACTACCCCTTCAAACCACCTAA
GGTTGCATTTACAACAAGAATTTATCATCCAAATATTAACAGTAATGGCA
GCATTTGTCTCGATATTCTAAGATCACAGTGGTCGCCTGCTTTAACAATT
TCTAAAGTTCTTTTATCCATTTGTTCACTGCTATGTGATCCAAACCCAGA
TGACCCCCTAGTGCCAGAGATTGCACGGATCTATAAAACAGACAGAGATA
AGTACAACAGAATATCTCGGGAATGGACTCAGAAGTATGCCATGTGAGCG
GCCGC
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