Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of UBE2D2

Enzyme description:- UBE2D2 2-147 (end)

Clone number:- DU20184

Source:- human recombinant

Tag:- cleaved from N-terminal His_6-tag

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

Expression system:- E.coli

Calculated molecular mass:-

Monoisotopic	18254 Da
Average Mass	18265 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 8.89

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

UBE2D2

Protein	UBE2D2 2-147 (end)
Synonyms	UbcH5b, E2D2
Clone Number	DU20184
Species	Human
Accession Number	Protein: P62837 DNA: NM_003339
Tags	N-terminal His_6 tag
Aminoacid sequence of the expressed protein	GSHMASMTGGQQMGRGS**ALKRIHKELNDLARDPPAQCSAGPVGDDMFHWQAT IMGPNDSPYQGGVFFLTIHFPTDYPFKPPKVAFTTRIYHPNINSNGSICLDI LRSQWSPALTISKVLLSICSLLCDPNPDDPLVPEIARIYKTDREKYNRIARE WTQKYAM**
Native sequence	Full length, but start Methionine is missing
Protease cleavage	Thrombin site underlined
Cloning sites	BamH1 / Not1

DNA sequence of insert

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GGATCCGCTCTGAAGAGAATCCACAAGGAATTGAATGATCTGGCACGGGA
CCCTCCAGCACAGTGTTCAGCAGGTCCTGTTGGAGATGATATGTTCCATT
GGCAAGCTACAATAATGGGGCCAAATGACAGTCCCTATCAGGGTGGAGTA
TTTTTCTTGACAATTCATTTCCCAACAGATTACCCCTTCAAACCACCTAA
GGTTGCATTTACAACAAGAATTTATCATCCAAATATTAACAGTAATGGCA
GCATTTGTCTTGATATTCTACGATCACAGTGGTCTCCAGCACTAACTATT
TCAAAAGTACTCTTGTCCATCTGTTCTCTGTTGTGTGATCCCAATCCAGA
TGATCCTTTAGTGCCTGAGATTGCTCGGATCTACAAAACAGATAGAGAAA
AGTACAACAGAATAGCTCGGGAATGGACTCAGAAGTATGCGATGTAAGCG
GCCGC
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