

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-UBE2D1

<u>Enzyme description:-</u>	UBE2D1 (2-147)
<u>Clone number:-</u>	DU4315
<u>Source:-</u>	human recombinant
<u>Tag:-</u>	N-terminal His ₆ -tag
<u>Purification method:-</u>	Ni ⁺⁺ -NTA-Sepharose
<u>Expression system:-</u>	<i>E.coli</i>

Calculated molecular mass:-

Monoisotopic	20001 Da
Average Mass	20014 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:- -80°C

Assay:-

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

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Clone Data Sheet

His-UBE2D1

<u>Protein</u>	UBE2D1 (2-147)
<u>Synonyms</u>	UbcH5a, E2D1, E2-17K-1
<u>Clone Number</u>	DU4315
<u>Species</u>	Human
<u>Accession Number</u>	Protein: P51668 DNA: NM_003338
<u>Tags</u>	N-terminal His ₆ tag
Aminoacid sequence of the expressed protein	MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSALKRIQKELSDLQRP PAHCSAGPVGDDLFWQATIMGPPDSAYQGGVFFLTVHFPTDYPFKPKI AFTTKIYHPNINSNGSICLDILRSQWSPALTVSKVLLSICSLLCDPNPDD PLVPDIAQIYKSDKEKYNRHAREWTQKYAM
Native sequence	in bold
Protease cleavage	Thrombin site underlined
Cloning sites	BamH1 / NotI

DNA sequence of insert

ATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCCTGGTGCCGCG
CGGCAGCCATATGGCTAGCATGACTGGTGGACAGCAAATGGGTTCGCGGAT
CCGCGCTGAAGAGGATTCAGAAAGAATTGAGTGATCTACAGCGCGATCCA
CCTGCTCACTGTTTCAGCTGGACCTGTGGGAGATGACTTGTTCCACTGGCA
AGCCACTATTATGGGGCCTCCTGATAGCGCATATCAAGGTGGAGTCTTCT
TTCTCACTGTACATTTTCCGACAGATTATCCTTTTAAACCACCAAAGATT
GCTTTCACAACAAAATTTACCATCCAAACATAAACAGTAATGGAAGTAT
TTGTCTCGATATTCTGAGGTCACAATGGTCACCAGCTCTGACTGTATCAA
AAGTTTTATTGTCCATATGTTCTCTACTTTGTGATCCTAATCCAGATGAC
CCTTAGTACCAGATATTGCACAAATCTATAAATCAGACAAAGAAAAATA
CAACAGACATGCAAGAGAATGGACTCAGAAATATGCAATGTAAGCGGCCG
C