

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-UBE2E3

Enzyme description:- His- UBE2E3 1-207 (full length)

Clone number:- DU14049

Source:- human recombinant

Tag:- N-terminal His₆-tag

Purification method:- Ni⁺⁺-NTA-Sepharose

Expression system:- *E.coli*

Calculated molecular mass:-

Monoisotopic 26539 Da

Average Mass 26554 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 9.07

Purity:- 90%

Enzyme storage buffer:-

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

Storage temperature:- -80°C

Assay:-

Loading assay with SUMO and SAE1/SAE2 in the presence of Mg-ATP

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Clone Data Sheet

His-UBE2E3

<u>Protein</u>	UBE2E3 1-207 (full length)
<u>Synonyms</u>	UbcH9, E2E3, UbcM2
<u>Clone Number</u>	DU14049
<u>Species</u>	Human
<u>Accession Number</u>	Protein: NP_006348
<u>Tags</u>	N-terminal His ₆ tag
Aminoacid sequence of the expressed protein	MGSSHHHHHSSGLVPRGSHMASMTGGQOMGRRSMSSDRQ RSDD ESPSTS SGSSDADQ RDPAAPEPEEQEERKPSATQ QK NTKLSSKTTAKLSTSAKRI QKELAEITL DPPNCSAGPKGDNIYEW RSTILGPPGS VYEGGVFFLDITF SSDY PFKPPKVTFR TR IYHCNINSQ GVICLDILKDN WSPAL TISKVLLSI CSLLTDCNPADPLV GS IATQYL TNRAEH DRIARQ WTKRYAT
Native sequence	in bold
Protease cleavage	Thrombin site underlined
Cloning sites	BamH1 / NotI
<u>DNA sequence of insert</u>	AGATCTATGTCCAGTGATAGGCAAAGGTCCGATGATGAGAGCCCCAGCAC CAGCAGTGGCAGTTCAGATGCGGACCAGCGAGACCCAGCCGCTCCAGAGC CTGAAGAACAAGAGGAAAGAAAACCTTCTGCCACCCAGCAGAAGAAAAAC ACCAAAC TCTCTAG CAAAACCACTGCTAAGTTATCCACTAGTGCTAAAAG AATTCAGAAGGAGCTAGCTGAAATAACCCCTTGATCCTCCTCCTAATTGCA GTGCTGGGCCTAAAGGAGATAACATTTATGAATGGAGATCAACTATACTT GGTCCACCGGGT TCTGT TATATGAAGGTGGTGTGTTTTTCTGGATATCAC ATTTTCATCAGATTATCCATTTAAGCCACCAAAGGTTACTTTCCGCACCA GAATCTATCACTGCAACATCAACAGTCAGGGAGTCATCTGTCTGGACATC CTTAAAGACAAC TGGAGT CCCGCTTTGACTATTTCAAAGGTTTTGCTGTC TATTTGTTCCCTTTTGACAGACTGCAACCC TGCGGAT CCTCTGGTTGGAA GCATAGCCACTCAGTATTTGACCAACAGAGCAGAACACGACAGGATAGCC AGACAGTGGACCAAGAGATACGCAACATAAGCGGCCGC