

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-UBE2E2

Enzyme description:- His-UBE2E2 (1-201) full length

Clone number:- DU12394

Source:- human recombinant

Tag:- N-terminal His₆-tag

Purification method:- Ni⁺⁺-NTA-Sepharose

Expression system:- *E.coli*

Calculated molecular mass:-

Monoisotopic 25651 Da

Average Mass 25667 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 with Ubiquitin and UBE1 in the presence of Mg-ATP

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Clone Data Sheet

His-UBE2E2

<u>Protein</u>	His-UBE2E2 (1-201) full length
<u>Synonyms</u>	UbcH8, E2E2
<u>Clone Number</u>	DU12394
<u>Species</u>	Human
<u>Accession Number</u>	Protein: NP_689866, Q96LR5
<u>Tags</u>	N-terminal His ₆ tag
Aminoacid sequence of the expressed protein	MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSM STE AQRVDDSPSTSG GSSDGDQ RESVQQEP EREQVQPKK EGKISSK TAAKLSTSAKRIQKELAE ITLDPPPNCSAGPKGDNIYEW RSTILGPPGS VYEGGVFFLDITFSPDY PF KPPKVTFRTRIYHCNINSQGVICLDILKDNWSPAL TISKVLLSIC SL LTDC CN PADPLVGS IATQYMTNRAEHDRMARQWTKRYAT
Native sequence	in bold
Protease cleavage	Thrombin site underlined
Cloning sites	BamH1 / NotI
<u>DNA sequence of insert</u>	GGATCCATGTCCACTGAGGCACAAAGAGTTGATGACAGTCCAAGCACTAG TGGAGGAAGTTCCGATGGAGATCAACGTGAAAGTGTTTCAGCAAGAACCAG AAAGAGAACAAGTTCAGCCCAAGAAAAAGGAGGGAAAAATATCCAGCAAA ACCGCTGCTAAATTGTCAACTAGTGCTAAAAGAATTCAGAAGGA ACTTGC AGAAATCACATTGGACCCTCCTCCCAACTGTAGTGCTGGACCCAAAGGAG ACAACATTTATGAATGGAGGTCAACTATATTGGGACCCCCAGGATCTGTC TATGAAGGAGGGGTGTTCTTTCTTGACATTACCTTTTACCAGACTATCC GTTTAAACCCCTAAGGTTACCTTCCGAACAAGAATCTATCACTGTAATA TTAACAGCCAAGGTGTGATCTGTCTGGACATCTTAAAGGACA ACTGGAGT CCGGCTTTAACTATTTCTAAAGTTCTCCTCTCCATCTGCTCACTTCTTAC AGATTGCAACCC TGCTG ACCCCTCTGGTGGGCAGCATCGCCACACAGTACA TGACCAACAGAGCAGAGCATGACCGGATGGCCAGACAGTGGACCAAGCGG TACGCCACATAGCGGCCGC