

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-UBE2D4

Enzyme description:- His-UBE2D4 (1-147)

Clone number:- DU8232

Source:- BL21 recombinant

Tag:- N-terminal His₆-tag

Purification method:- Ni⁺⁺-NTA-Sepharose

Expression system:- *E.coli*

Calculated molecular mass:-

Monoisotopic 22398 Da

Average Mass 22412 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 8.8

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-UBE2D4

<u>Protein</u>	His-UBE2D4 (1-147)
<u>Synonyms</u>	HBUCE1, UbcH5D
<u>Clone Number</u>	DU8232
<u>Species</u>	Human
<u>Accession Number</u>	Protein: Q9Y2X8
<u>Tags</u>	N-terminal His ₆ tag
Aminoacid sequence of the expressed protein	MGSSHHHHHSSGLVPRGSHMASMTGGQOMGRGSEFELGSTSNGRQCAGI RPCAAAMALKRIQKELTDLQRPQAQCSAGPVGDDLFWQATIMGPNDSP YQGGVFFLTIHFPTDYPFKPPKVAFTTKIYHPNINSNGSICLDILRSQWS PALTVSKVLLSICSLLCDPNPDDPLVPEIAHTYKADREKYNRLAREWTQK YAM
Native sequence	in bold
Protease cleavage	Thrombin site underlined
Cloning sites	Not1
<u>DNA sequence of insert</u>	GCGGCCGCGATGGCGCTAAAGCGGATCCAGAAGGAATTAACCGACTTGCA GAGGGATCCTCCTGCCAGTGTTCCTGCAGGACCTGTCGGTGATGACTTGT TCCACTGGCAGGCCACCATCATGGGCCCGAATGACAGTCCCTTACCAAGGA GGTGTTCCTCCTGACCATCCACTTTCCTACAGATTACCCGTTCAAGCC CCCAAAGGTTGCTTTCACAACCAAAATTTATCACCCCTAATATCAACAGCA ATGGCAGCATCTGCCTTGATATCCTGCGGTCTCAGTGGTCTCCAGCGTTG ACTGTGTCAAAAAGTTCTTGTCCATCTGCTCGCTGCTCTGCGACCCCAA CCCCGATGACCCCTGGTGCCAGAGATAGCACACACCTACAAGGCCGACA GAGAGAAGTACAACAGACTAGCAAGAGAGTGGACACAAAAATATGCTATG TAAGCGGCCGC