

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of UBE2D4

<u>Enzyme description:-</u>	UBE2D4 (1-147)
<u>Clone number:-</u>	DU8232
<u>Source:-</u>	human recombinant
<u>Tag:-</u>	cleaved from N-terminal His ₆ -tag
<u>Purification method:-</u>	Ni ⁺⁺ -NTA-Sepharose, Thrombin treatment, SEC
<u>Expression system:-</u>	<i>E.coli</i>
<u>Calculated molecular mass:-</u>	
Monoisotopic	20518 Da
Average Mass	20530 Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	8.46
<u>Purity:-</u>	90%
<u>Enzyme storage buffer:-</u>	
50mM HEPES pH 7.5, 150mM NaCl, 10% glycerol, 1mM DTT	
<u>Storage temperature:-</u>	-80°C
<u>Assay:-</u>	
Loading with Ubiquitin and UBE1 in the presence of Mg-ATP	

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Clone Data Sheet

UBE2D4

Protein UBE2D4 (1-147)
Synonyms HBUCE1, UbcH5D
Clone Number DU8232
Species Human
Accession Number Protein: Q9Y2X8
Tags N-terminal His₆ tag
Aminoacid sequence of the expressed protein **G****S****H****M****A****S****M****T****G****Q****Q****M****G****R****G****S****E****F****E****L****G****S****T****S****N****G****R****Q****C****A****G****I****R****P****C****A****A****M****A****L****K****R****I****Q****K****E****L****T****D****L**
Q**R****D****P****P****A****Q****C****S****A****G****P****V****G****D****D****L****F****H****W****Q****A****T****I****M****G****P****N****D****S****P****Y****Q****G****G****V****F****F****L****T****I****H****F****P****T****D****Y****P****F****K****P**
K**V****A****F****T****T****K****I****Y****H****P****N****I****N****S****N****G****S****I****C****L****D****I****L****R****S****Q****W****S****P****A****L****T****V****S****K****V****L****L****S****I****C****S****L****L****C****D****P****N****P****D**
P**L****V****P****E****I****A****H****T****Y****K****A****D****R****E****K****Y****N****R****L****A****R****E****W****T****Q****K****Y****A****M**

Native sequence in bold
Protease cleavage Thrombin site underlined
Cloning sites Not1

DNA sequence of insert GCGGCCGCGATGGCGCTAAAGCGGATCCAGAAGGAATTAACCGACTTGCA
GAGGGATCCTCCTGCCAGTGTTCCTGCAGGACCTGTCCGGTGATGACTTGT
TCCACTGGCAGGCCACCATCATGGGCCCGAATGACAGTCCCTTACCAAGGA
GGTGTTCCTTCTCCTGACCATCCACTTTCCTACAGATTACCCGTTCAAGCC
CCCAAAGGTTGCTTTCACAACCAAAATTTATCACCCCTAATATCAACAGCA
ATGGCAGCATCTGCCTTGATATCCTGCGGTCTCAGTGGTCTCCAGCGTTG
ACTGTGTCAAAAAGTTCTTGTCCATCTGCTCGCTGCTCTGCGACCCCAA
CCCCGATGACCCCTGGTGCCAGAGATAGCACACACCTACAAGGCCGACA
GAGAGAAGTACAACAGACTAGCAAGAGAGTGGACACAAAAATATGCTATG
TAAGCGGCCGC