

## *Division of Signal Transduction Therapy*

### **Standard Operation Procedure**

#### **Preparation of UBE2D4**

<b><u>Enzyme description:-</u></b>	UBE2D4
<b><u>Clone number:-</u></b>	DU8232
<b><u>Source:-</u></b>	BL21 recombinant
<b><u>Tag:-</u></b>	cleaved from N-terminal His <sub>6</sub> -tag
<b><u>Purification method:-</u></b>	Ni <sup>++</sup> -NTA-Sepharose, Thrombin treatment, SEC
<b><u>Expression level:-</u></b>	15mg/L
<b><u>Calculated molecular mass:-</u></b>	
Monoisotopic	20518 Da
Average Mass	20530 Da
[cysteines reduced, methionines have not been oxidised]	
<b><u>Theoretical pI:-</u></b>	8.46
<b><u>Purity:-</u></b>	90%
<b><u>Enzyme storage buffer:-</u></b>	
50mM HEPES pH 7.5, 150mM NaCl, 10% glycerol, 1mM DTT	
<b><u>Storage temperature:-</u></b>	-80°C
<b><u>Assay:-</u></b>	
Loading with Ubiquitin and UBE1 in the presence of Mg-ATP	

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### **Clone Data Sheet**

#### **UBE2D4**

<b><u>Protein</u></b>	UBE2D4
<b><u>Synonyms</u></b>	HBUCE1, UbcH5D
<b><u>Clone Number</u></b>	DU8232
<b><u>Species</u></b>	Human
<b><u>Accession Number</u></b>	Protein: Q9Y2X8
<b><u>Tags</u></b>	N-terminal His <sub>6</sub> tag
Aminoacid sequence of the expressed protein	<b>GSHMASMTGGQOMGRGSEFELGSTSNGRQCAGIRPCAAAMALKRIQKELTDL QRDPPAQCSAGPVGDDLFWQATIMGPNDSPYQGGVFFLTIHFPTDYPFKPP KVAFTTKIYHPNINSNGSICLDILRSQWSPALTVSKVLLSICSLLCDPNPDD PLVPEIAHTYKADREKYNRLAREWTQKYAM</b>
Native sequence	in bold
Protease cleavage	Thrombin site underlined
Cloning sites	Not1

**DNA sequence of insert**

GCGGCCGCGATGGCGCTAAAGCGGATCCAGAAGGAATTAACCGACTTGCA  
GAGGGATCCTCCTGCCCAGTGTCTGCAGGACCTGTTCGGTGATGACTTGT  
TCCACTGGCAGGCCACCATCATGGGCCCGAATGACAGTCCTTACCAAGGA  
GGTGTTCCTTCTTCCATCCACTTTCCTACAGATTACCCGTTCAAGCC  
CCCAAAGGTTGCTTTCACAACCAAAATTTATCACCTAATATCAACAGCA  
ATGGCAGCATCTGCCTTGATATCCTGCGGTCTCAGTGGTCTCCAGCGTTG  
ACTGTGTCAAAGTTCTCTTGTCATCTGCTCGCTGCTCTGCGACCCCAA  
CCCCGATGACCCCTGGTGCCAGAGATAGCACACACCTACAAGGCCGACA  
GAGAGAAGTACAACAGACTAGCAAGAGAGTGGACACAAAATATGCTATG  
TAAGCGGCCGC