

## *Division of Signal Transduction Therapy*

### **Standard Operation Procedure**

#### **Preparation of His-UBE2N**

<b><u>Enzyme description:-</u></b>	His-UBE2N
<b><u>Clone number:-</u></b>	DU15705
<b><u>Source:-</u></b>	BL21 recombinant
<b><u>Tag:-</u></b>	N-terminal His <sub>6</sub> -tag
<b><u>Purification method:-</u></b>	Ni <sup>++</sup> -NTA-Sepharose
<b><u>Expression level:-</u></b>	9mg/L
<b><u>Calculated molecular mass:-</u></b>	
Monoisotopic	19439 Da
Average Mass	19450Da
[cysteines reduced, methionines have not been oxidised]	
<b><u>Theoretical pI:-</u></b>	6.87
<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

#### **Assay:-**

Production of free K63 linked Ub-chains with Ubiquitin, UBE1 and UBE2V1 in the presence of Mg-ATP

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**Clone Data Sheet**

**His-UBE2N**

<b><u>Protein</u></b>	UBE2N
<b><u>Synonyms</u></b>	Ubc13, BLU
<b><u>Clone Number</u></b>	DU15705
<b><u>Species</u></b>	Human
<b><u>Accession Number</u></b>	Protein: P61088      DNA: NM_003348
<b><u>Tags</u></b>	N-terminal His <sub>6</sub> tag
Aminoacid sequence of the expressed protein	MGSSHHHHHHSSGLEVL <u>FQ</u> PGSAGLPRRI <b>IKETQ</b> RLLAEPVPGIKAEPD <b>ESNARYFHVVIAGPQDSPFEGGTFKLELFLPEEYPMAAPKVRFMTKIYHP</b> <b>NVDKLGRI</b> CLDILKDKWSPALQ <b>IRTVLLSIQALLSAPNPDDPLANDVAEQ</b> <b>WKTNEAQAIETARAWTRLYAMNNI</b>
Native sequence	in bold, Start Met is missing
Protease cleavage	Prescission site underlined
Cloning sites	BamH1 / EcoRI
<b><u>DNA sequence of insert</u></b>	GGATCCGCCGGGCTGCCCGCAGGATCATCAAGGAAACCCAGCGTTTGCT GGCAGAACCAGTTCCTGGCATCAAAGCCGAACCAGATGAGAGCAACGCC GTTATTTTTCATGTGGTCATTGCTGGCCCTCAGGATTCCCCCTTTGAGGGA GGGACTTTTAAACTTGAACATTCCTTCCAGAAGAATACCCAATGGCAGC CCCTAAAGTACGTTTCATGACCAAAATTTATCATCCTAATGTAGACAAGT TGGGAAGAATATGTTTAGATATTTTGAAAGATAAGTGGTCCCCAGCACTG CAGATCCGCACAGTTCCTGCTATCGATCCAGGCCTTGTTAAGTGCTCCCAA TCCAGATGATCCATTAGCAAATGATGTAGCGGAGCAGTGGAAGACCAACG AAGCCCAAGCCATAGAAACAGCTAGAGCATGGACTAGGCTATATGCCATG AATAATATTTAAGAATTC