

## *Division of Signal Transduction Therapy*

### **Standard Operation Procedure**

#### **Preparation of UBE2V2**

<b><u>Enzyme description:-</u></b>	UBE2V2 (1-145) full length
<b><u>Clone number:-</u></b>	DU12415
<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, protease treatment, SEC
<b><u>Expression level:-</u></b>	6mg/L
<b><u>Calculated molecular mass:-</u></b>	
Monoisotopic	16650 Da
Average Mass	16660 Da
[cysteines reduced, methionines have not been oxidised]	
<b><u>Theoretical pI:-</u></b>	8.8
<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 UBE2N in the presence of Mg-ATP

Assay buffer: 50mM HEPES, pH 7.5, 1mM DTT

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**Clone Data Sheet**

**His-UBE2V2**

**Protein** UBE2V2  
**Synonyms** UEV2, MMS-2  
**Clone Number** DU12415  
**Species** Human  
**Accession Number** Protein: NP\_003341      DNA: NM\_003350  
**Tags** N-terminal His<sub>6</sub> tag (cleaved)  
Aminoacid sequence of the expressed protein **GPGSMAVSTG**V**KVPRN**F**RLLEELEEG**Q**KG**V**GD**G**TV**S**W**G**LE**D**ED**M**TL**T**R**W**T**G**  
**MI**IG**P**PR**T**NY**E**NR**I**Y**S**L**K**VE**C**GP**K**Y**P**E**A**PP**S**VR**F**V**T**K**I**N**M**NG**I**NN**S**S**G**M**V**DA  
**RS**IP**V**L**A**K**W**Q**N**S**Y**S**I**K**V**VL**Q**EL**R**RL**M**MS**K**EN**M**K**L**P**Q**PE**G**Q**T**Y**N**  
Native sequence in bold  
Protease cleavage Precission site underlined  
Cloning sites BamH1 / Not1**

**DNA sequence of insert**  
GGATCCATGGCGGTCTCCACAGGAGTTAAAGTTCCTCGTAATTTTCGCTT  
GTTGGAAGAACTTGAAGAAGGACAAAAAGGAGTAGGCGACGGTACAGTTA  
GCTGGGGCCTTGAAGATGATGAAGATATGACACTTACAAGGTGGACAGGC  
ATGATTATTGGGCCACCAAGGACAAATTATGAAAACAGAATATATAGCCT  
GAAAGTAGAATGTGGACCTAAATACCCAGAAGCTCCTCCGTCAGTTAGAT  
TTGTAACAAAAATTAATATGAACGGAATAAATAATTCCAGTGGGATGGTG  
GATGCCCGGAGCATACCAAGTGTAGCAAAATGGCAAAATTCATATAGCAT  
TAAAGTTGTACTTCAAGAGCTAAGACGTCTAATGATGTCCAAAAGAAATA  
TGAAGCTTCCACAGCCACCAGAAGGACAAACATACAACAATTAAGCGGCC  
GC