

## *Division of Signal Transduction Therapy*

### **Standard Operation Procedure**

#### **Preparation of UBE2D3**

<b><u>Enzyme description:-</u></b>	UBE2D3 2-147 (end)
<b><u>Clone number:-</u></b>	DU15703
<b><u>Source:-</u></b>	human 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
<b><u>Expression level:-</u></b>	E.coli
<b><u>Calculated molecular mass:-</u></b>	
Monoisotopic	16843 Da
Average Mass	16853 Da
[cysteines reduced, methionines have not been oxidised]	
<b><u>Theoretical pI:-</u></b>	8.54
<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**

**UBE2D3**

**Protein** UBE2D3 2-147 (end)  
**Synonyms** UbcH5c, E2D3  
**Clone Number** DU15703  
**Species** Human  
**Accession Number** Protein: P61077      DNA: NM\_003340  
**Tags** cleaved from N-terminal His<sub>6</sub> tag  
Aminoacid sequence of the purified protein GPGSALKRINKELSDLARDPPAQCSAGPVGDDMFHWQATIMGPNDSPYQGGV  
**FFLTIHFPTDYPFKPPKVAFTTRIYHPNINSNGSICLDILRSQWSPALTIK**  
**VLLSICSLLCDPNPDDPLVPEIARIYKTRDKYNRISREWTQKYAM**  
Native sequence Start Methionine is missing  
Protease cleavage Prescission site underlined  
Cloning sites BamH1 / Not1  
  
**DNA sequence of insert** GGATCCGCGCTGAAACGGATTAATAAGGAACTTAGTGATTTGGCCCGTGA  
CCCTCCAGCACAAATGTTCTGCAGGTCCAGTTGGGGATGATATGTTTCATT  
GGCAAGCCACAATTATGGGACCTAATGACAGCCCATATCAAGGCGGTGTA  
TTCTTTTTGACAATTCATTTTCCTACAGACTACCCCTTCAAACCACCTAA  
GGTTGCATTTACAACAAGAATTTATCATCAAATATTAACAGTAATGGCA  
GCATTTGTCTCGATATTCTAAGATCACAGTGGTCGCCTGCTTTAACAATT  
TCTAAAGTTCTTTTATCCATTTGTTCACTGCTATGTGATCCAAACCCAGA  
TGACCCCTAGTGCCAGAGATTGCACGGATCTATAAAACAGACAGAGATA  
AGTACAACAGAATATCTCGGGAATGGACTCAGAAGTATGCCATGTGAGCG  
GCCGC