

## *Division of Signal Transduction Therapy*

### **Standard Operation Procedure**

#### **Preparation of UBE2E3**

<b><u>Enzyme description:-</u></b>	UBE2E3 1-207 (full length)
<b><u>Clone number:-</u></b>	DU14049
<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, Thrombin treatment, SEC
<b><u>Expression system:-</u></b>	<i>E.coli</i>
<b><u>Calculated molecular mass:-</u></b>	
Monoisotopic	24568 Da
Average Mass	24672 Da
[cysteines reduced, methionines have not been oxidised]	
<b><u>Theoretical pI:-</u></b>	8.84
<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:-**

Loading assay with SUMO and SAE1/SAE2 in the presence of Mg-ATP

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**Clone Data Sheet**

**His-UBE2E3**

**Protein** UBE2E3 1-207 (full length)  
**Synonyms** UbcH9, E2E3, UbcM2  
**Clone Number** DU14049  
**Species** Human  
**Accession Number** Protein: NP\_006348  
**Tags** N-terminal His<sub>6</sub> tag  
Aminoacid sequence of the expressed protein **G**SHMASMTGGQQMGRRS**MSSDRQ**RSDD**ESPSTSSGSSDADQRDPAAPEPEEQ**  
**EERKPSATQOKKNTKLSSKTTAKLSTSAKRIQELAEITLDPPPNC**SAG**PKG**  
**DNIY**EW**RSTILGPPG**SVYEGGV**FFLDITFSSDYPFKPPKVTFRTRI**YHC**NI**  
**SQGVICLDILKDNWSPALTI**SKV**LLSICSL**LLTDCNPAD**PLVGS**IATQ**YLTNR**  
**AEHDRIARQWTKRYAT**  
Native sequence in bold  
Protease cleavage Thrombin site underlined  
Cloning sites BamH1 / NotI

**DNA sequence of insert** AGATCTATGTCCAGTGATAGGCAAAGGTCCGATGATGAGAGCCCCAGCAC  
CAGCAGTGGCAGTTCAGATGCGGACCAGCGAGACCCAGCCGCTCCAGAGC  
CTGAAGAACAAGAGGAAAGAAAACCTTCTGCCACCCAGCAGAAGAAAAAC  
ACCAAACCTCTCTAGCAAAACCACTGCTAAGTTATCCACTAGTGCTAAAAG  
AATTCAGAAGGAGCTAGCTGAAATAACCCCTTGATCCTCCTCCTAATTGCA  
GTGCTGGGCCTAAAGGAGATAACATTTATGAATGGAGATCAACTATACTT  
GGTCCACCGGGTTCTGTATATGAAGGTGGTGTGTTTTTCTGGATATCAC  
ATTTTCATCAGATTATCCATTTAAGCCACCAAAGGTTACTTTCGGCACCA  
GAATCTATCACTGCAACATCAACAGTCAGGGAGTCATCTGTCTGGACATC  
CTTAAAGACAACCTGGAGTCCCGCTTTGACTATTTCAAAGGTTTTGCTGTC  
TATTTGTTCCCTTTTGACAGACTGCAACCCCTGCGGATCCTCTGGTTGGAA  
GCATAGCCACTCAGTATTTGACCAACAGAGCAGAACACGACAGGATAGCC  
AGACAGTGGACCAAGAGATACGCAACATAAGCGGCCGC