

## *Division of Signal Transduction Therapy*

### **Standard Operation Procedure**

#### **Preparation of UBE2E2**

|   |   |
|---|---|
| <b><u>Enzyme description:-</u></b>                      | UBE2E2 (1-201) full length                    |
| <b><u>Clone number:-</u></b>                            | DU12394                                       |
| <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               |
| <b><u>Expression system:-</u></b>                       | <i>E.coli</i>                                 |
| <b><u>Calculated molecular mass:-</u></b>               |   |
| Monoisotopic  | 23901 Da                                      |
| Average Mass  | 23916 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   |
| <b><u>Assay:-</u></b>                                   |   |

Loading with Ubiquitin and UBE1 in the presence of Mg-ATP

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**Clone Data Sheet**

**UBE2E2**

**Protein** UBE2E2 (1-201) full length  
**Synonyms** UbcH8, E2E2  
**Clone Number** DU12394  
**Species** Human  
**Accession Number** Protein: NP\_689866, Q96LR5  
**Tags** cleaved from N-terminal His<sub>6</sub> tag  
Aminoacid sequence of the expressed protein GSHMASMTGGQQMGRGSM**STEAQRVDDSPSTSGGSSDGDQRESVQQEPEREQ**  
**VQPKKKEGKISSKTAAKLSTSAKRIQKELAEITLDPPPNC**SAGPKGDNIYEW  
**RSTILGPPGSVYEGGVFFLDITFSPDYPFKPPKVTFRTRIYHCNINSQVIC**  
**LDILKDNWSPALTI**SKVLLSICSLLLTDCNPADPLVGS IATQYMTNRAEHRM  
**ARQWTKRYAT**  
Native sequence in bold  
Protease cleavage Thrombin site underlined  
Cloning sites BamH1 / NotI

**DNA sequence of insert** GGATCCATGTCCACTGAGGCACAAAGAGTTGATGACAGTCCAAGCACTAG  
TGGAGGAAGTTCCGATGGAGATCAACGTGAAAGTGTTTCAGCAAGAACCAG  
AAAGAGAACAAGTTCAGCCCAAGAAAAAGGAGGGAAAAATATCCAGCAAA  
ACCGCTGCTAAATTGTCAACTAGTGCTAAAAGAATTCAGAAGGAACTTGC  
AGAAATCACATTGGACCCTCCTCCCAACTGTAGTGCTGGACCCAAAGGAG  
ACAACATTTATGAATGGAGGTCAACTATATTGGGACCCCCAGGATCTGTC  
TATGAAGGAGGGGTGTTCTTTCTTGACATTACCTTTTACCAGACTATCC  
GTTTAAACCCCTAAGGTTACCTTCCGAACAAGAATCTATCACTGTAATA  
TTAACAGCCAAGGTGTGATCTGTCTGGACATCTTAAAGGACAACCTGGAGT  
CCGGCTTTAACTATTTCTAAAGTTCTCCTCTCCATCTGCTCACTTCTTAC  
AGATTGCAACCCTGCTGACCCTCTGGTGGGCAGCATCGCCACACAGTACA  
TGACCAACAGAGCAGAGCATGACCGGATGGCCAGACAGTGGACCAAGCGG  
TACGCCACATAGCGGCCGC