

## *Division of Signal Transduction Therapy*

### **Standard Operation Procedure**

#### **Preparation of UBE2D1**

<b><u>Enzyme description:-</u></b>	UBE2D1 (2-147)
<b><u>Clone number:-</u></b>	DU4315
<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, SEC
<b><u>Expression system:-</u></b>	<i>E.coli</i>
<b><u>Calculated molecular mass:-</u></b>	
Monoisotopic	18120 Da
Average Mass	18132 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**

**His-UBE2D1**

<b><u>Protein</u></b>	UBE2D1 (2-147)
<b><u>Synonyms</u></b>	UbcH5a, E2D1, E2-17K-1
<b><u>Clone Number</u></b>	DU4315
<b><u>Species</u></b>	Human
<b><u>Accession Number</u></b>	Protein: P51668    DNA: NM_003338
<b><u>Tags</u></b>	N-terminal His <sub>6</sub> tag, cleaved
Aminoacid sequence of the expressed protein	<b><u>G</u>SHMASMTGGQQMGRGS<b><u>A</u></b>LK<b><u>R</u></b>IQ<b><u>K</u></b>ELSD<b><u>L</u></b>QR<b><u>D</u></b>PP<b><u>A</u></b>HC<b><u>S</u></b>AG<b><u>P</u></b>V<b><u>G</u></b>DD<b><u>L</u></b>F<b><u>H</u></b>W<b><u>Q</u></b>A<b><u>T</u></b> <b><u>I</u></b>M<b><u>G</u></b>P<b><u>P</u></b>D<b><u>S</u></b>A<b><u>Y</u></b>Q<b><u>G</u></b>G<b><u>V</u></b>F<b><u>F</u></b>L<b><u>T</u></b>V<b><u>H</u></b>F<b><u>P</u></b>T<b><u>D</u></b>Y<b><u>P</u></b>F<b><u>K</u></b>P<b><u>P</u></b>K<b><u>I</u></b>A<b><u>F</u></b>T<b><u>T</u></b>K<b><u>I</u></b>Y<b><u>H</u></b>P<b><u>N</u></b>I<b><u>N</u></b>S<b><u>N</u></b>G<b><u>S</u></b>I<b><u>C</u></b>L<b><u>D</u></b>I <b><u>L</u></b>R<b><u>S</u></b>Q<b><u>W</u></b>S<b><u>P</u></b>A<b><u>L</u></b>T<b><u>V</u></b>S<b><u>K</u></b>V<b><u>L</u></b>L<b><u>S</u></b>I<b><u>C</u></b>S<b><u>L</u></b>L<b><u>C</u></b>D<b><u>P</u></b>N<b><u>P</u></b>D<b><u>D</u></b>P<b><u>L</u></b>V<b><u>P</u></b>D<b><u>I</u></b>A<b><u>Q</u></b>I<b><u>Y</u></b>K<b><u>S</u></b>D<b><u>K</u></b>E<b><u>K</u></b>Y<b><u>N</u></b>R<b><u>H</u></b>A<b><u>R</u></b>E <b><u>W</u></b>T<b><u>Q</u></b>K<b><u>Y</u></b>A<b><u>M</u></b></b>
Native sequence	in bold
Protease cleavage	Thrombin site underlined
Cloning sites	BamH1 / NotI
<b><u>DNA sequence of insert</u></b>	ATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCCTGGTGCCGCG CGGCAGCCATATGGCTAGCATGACTGGTGGACAGCAAATGGGTTCGCGGAT CCGCGCTGAAGAGGATTCAGAAAGAATTGAGTGATCTACAGCGCGATCCA CCTGCTCACTGTTTCAGCTGGACCTGTGGGAGATGACTTGTTCCTACTGGCA AGCCACTATTATGGGGCCTCCTGATAGCGCATATCAAGGTGGAGTCTTCT TTCTCACTGTACATTTTCCGACAGATTATCCTTTTAAACCACCAAAGATT GCTTTCACAACAAAAATTTACCATCCAAACATAAACAGTAATGGAAGTAT TTGTCTCGATATTCTGAGGTCACAATGGTCACCAGCTCTGACTGTATCAA AAGTTTTATTGTCCATATGTTCTCTACTTTGTGATCCTAATCCAGATGAC CCCTTAGTACCAGATATTGCACAAATCTATAAATCAGACAAAGAAAAATA CAACAGACATGCAAGAGAATGGACTCAGAAATATGCAATGTAAGCGGCCG C