

## *Division of Signal Transduction Therapy*

### **Standard Operation Procedure**

#### **Preparation of UBE2S**

<b><u>Enzyme description:-</u></b>	UBE2S
<b><u>Clone number:-</u></b>	DU20175
<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
<b><u>Expression level:-</u></b>	3mg/L
<b><u>Calculated molecular mass:-</u></b>	
Monoisotopic	25360 Da
Average Mass	25374 Da
[cysteines reduced, methionines have not been oxidised]	
<b><u>Theoretical pI:-</u></b>	9.37
<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

#### UBE2S

<b><u>Protein</u></b>	UBE2S
<b><u>Synonyms</u></b>	E2-EPF, EPF5
<b><u>Clone Number</u></b>	DU20175
<b><u>Species</u></b>	Human
<b><u>Accession Number</u></b>	Protein: NP_055316, DNA: NM_014501
<b><u>Tags</u></b>	N-terminal His <sub>6</sub> tag (cleaved)
Aminoacid sequence of the purified protein	<b>GSHMASMTGGQOMGRGSNSNVENLPPHIIRLVYKEVTTLTADPPDGIKVFPN EEDLTDLQVTIEGPEGTPYAGGLFRMKLLLGKDFPASPPKGYFLTKIFHPNV GANGEICVNVLKRDWTAELGIRHVLLTIKCLLIHPNPESALNEEAGRLLLEN YEEYAARARLLTEIHGGAGGPPSGRAEAGRALASGTEASSTDPGAPGGPGGAE GPMAKKHAGERDKKLAAKKTKDKKRALRRL</b>
Native sequence	in bold, Start Met is missing
Protease cleavage	Thrombin site underlined
Cloning sites	BamH1 / NotI
<b><u>DNA sequence of insert</u></b>	<b><u>GGATCCA</u>ACTCCAACGTGGAGAACCTACCCCCGCACATCATCCGCCTGG TGTACAAGGAGGTGACGACACTGACCGCAGACCCACCCGATGGCATCAAG GTCTTTCCCAACGAGGAGGACCTCACCGACCTCCAGGTCACCATCGAGGG CCCTGAGGGGACCCCATATGCTGGAGGTCTGTTCCGCATGAAACTCCTGC TGGGGAAGGACTTCCTGCCTCCCCACCCAAGGGCTACTTCCTGACCAAG ATCTTCCACCCGAACGTGGGCGCCAATGGCGAGATCTGCGTCAACGTGCT CAAGAGGGACTGGACGGCTGAGCTGGGCATCCGACACGTACTGCTGACCA TCAAGTGCCTGCTGATCCACCCTAACCCGAGTCTGCACTCAACGAGGAG GCGGGCCGCTGCTCTTGGAGAACTACGAGGAGTATGCAGCTCGGGCCCG TCTGCTCACAGAGATCCACGGGGCGCCGGCGGGCCCAGCGGCAGGGCCG AAGCCGGTCGGGCCCTGGCCAGTGGCACTGAAGCTTCCTCCACCGACCCT GGGGCCCCAGGGGGCCCGGGAGGGGCTGAGGGTCCCATGGCCAAGAAGCA TGCTGGCGAGCGGATAAGAAGCTGGCGGCAAGAAAAAGACGGACAAGA AGCGGGCGCTGCGGCGGCTGTAGGCGGCCGC</b>