

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-UBE2S

<u>Enzyme description:-</u>	UBE2S
<u>Clone number:-</u>	DU20175
<u>Source:-</u>	BL21 recombinant
<u>Tag:-</u>	N-terminal His ₆ -tag
<u>Purification method:-</u>	Ni ⁺⁺ -NTA-Sepharose
<u>Expression level:-</u>	4mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	26878 Da
Average Mass	26894 Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	9.62
<u>Purity:-</u>	90%
<u>Enzyme storage buffer:-</u>	
50mM HEPES pH 7.5, 150mM NaCl, 10% glycerol, 1mM DTT	
<u>Storage temperature:-</u>	-80°C
<u>Assay:-</u>	
Loading with Ubiquitin and UBE1 in the presence of Mg-ATP	

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Clone Data Sheet

His-UBE2S

<u>Protein</u>	UBE2S
<u>Synonyms</u>	E2-EPF, EPF5
<u>Clone Number</u>	DU20175
<u>Species</u>	Human
<u>Accession Number</u>	Protein: NP_055316, DNA: NM_014501
<u>Tags</u>	N-terminal His ₆ tag
Aminoacid sequence of the expressed protein	HHHHHSSGLVPRGSHMASMTGGQQMGRGSNSNVENLPPHIIRLVYKEVT TLTADPPDGIKVFPNEEDLTDLQVTIEGPEGTPYAGGLFRMKLLLGKDFP ASPPKGYFLTKIFHPNVGANGEICVNVLKRWDTAELGIRHVLLTIKLLI HPNPESALNEEAGRLLLENYEEYAARARLLTEIHGGAGGPPSGRAEAGRAL ASGTEASSTDPGAPGGPGEAEGPMAKKHAGERDKKLAAKKTKDKRALRR L
Native sequence	in bold, Start Met is missing
Protease cleavage	Thrombin site underlined
Cloning sites	BamH1 / NotI
<u>DNA sequence of insert</u>	<u>GGATCCA</u> ACTCCAACGTGGAGAACCTACCCCCGCACATCATCCGCCTGG TGTACAAGGAGGTGACGACACTGACCGCAGACCCACCCGATGGCATCAAG GTCTTTCCAACGAGGAGGACCTCACCGACCTCCAGGTCAACATCGAGGG CCCTGAGGGGACCCATATGCTGGAGGTCTGTTCCGCATGAAACTCCTGC TGGGGAAGGACTTCCCTGCCTCCCCACCAAGGGCTACTTCCCTGACCAAG ATCTTCCACCCGAACGTGGGCGCCAATGGCGAGATCTGCGTCAACGTGCT CAAGAGGACTGGACGGCTGAGCTGGGCATCCGACACGTACTGCTGACCA TCAAGTGCCTGCTGATCCACCCTAACCCGAGTCTGCACTCAACGAGGAG GCGGGCCGCTGCTCTTGGAGAACTACGAGGAGTATGCAGCTCGGGCCG TCTGCTCACAGAGATCCACGGGGCGCCGGCGGGCCCAGCGGCAGGGCCG AAGCCGGTCGGGCCCTGGCCAGTGGCACTGAAGCTTCCCTCCACCGACCCT GGGGCCCAGGGGCCCAGGAGGGGCTGAGGGTCCCATGGCCAAGAAGCA TGCTGGCGAGCGGATAAGAAGCTGGCGGCAAGAAAAAGACGGACAAGA AGCGGGCGCTGCGGCGGCTGTAGGCGGCCG