

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of UBE2C

<u>Enzyme description:-</u>	UBE2C = UbcH10 1-179 (full length)
<u>Clone number:-</u>	DU32146
<u>Source:-</u>	human recombinant
<u>Tag:-</u>	cleaved from N-terminal His ₆ -tag
<u>Purification method:-</u>	Ni ⁺⁺ -NTA-Sepharose, protease treatment
<u>Expression system:-</u>	E.coli
<u>Calculated molecular mass:-</u>	
Monoisotopic	19938 Da
Average Mass	19949 Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	7.7
<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	

Division of Signal Transduction Therapy

Clone Data Sheet

UBE2C

<u>Protein</u>	UBE2C 1-179 (full length)
<u>Synonyms</u>	UbcH10
<u>Clone Number</u>	DU32146
<u>Species</u>	Human
<u>Accession Number</u>	Protein: NP_009850
<u>Tags</u>	cleaved from N-terminal His ₆ tag
Aminoacid sequence of the expressed protein	<u>GP</u>GS<u>MASQ</u>NRDPAATS<u>VAAARKGAEP</u>SGGAARGPVGKRL<u>QQ</u>ELMTLM<u>MSGDKG</u>ISAF<u>PESDNL</u>FKWVG<u>TIHGAAGTV</u>YEDLRYKLSLE<u>FPSGYP</u><u>YNAPT</u>VK<u>FLTPCY</u>HPNVDT<u>QGNICLD</u>ILKEKWSALYD<u>VRTILL</u>SI<u>QS</u><u>LLGEP</u>NI<u>DSPLN</u>THAAELWKNPTAFK<u>YLQ</u>ETYSK<u>QVTSQ</u>EP
Native sequence	
Protease cleavage	Prescission site underlined
Cloning sites	BamH1 / Not1
<u>DNA sequence of insert</u>	GGATCCATGGCTTCCCAAACCGCGACCCAGCCG <u>CCACTAGCGTCGC</u> CGCCGCCCGTAAAGGAGCTGAGCCGAGCGGGGGCGCCGCCCGGGGTC CGGTGGGCAAAGGCTACAGCAGGAGCTGATGACCC <u>TCATGATGTCT</u> GGCGATAAAGGATTTCTGCCTTCCCTGAATCAGACAACCTTTTCAA ATGGGTAGGGACCATCCATGGAGCAGCTGGAACAGTATATGAAGACC TGAGGTATAAGCTCTCGCTAGAGTTCCCCAGTGGCTACCC <u>TTACAAT</u> GCGCCACAGTGAAGTTCC <u>TCACGCC</u> CTGCTATCACCCCAACGTGGA CACCCAGGGTAACATATGCCTGGACATCCTGAAGGAAAAGTGGTCTG CCCTGTATGATGTCAGGACCATTCTGCTCTCCATCCAGAGCCTTCTA GGAGAACCCAACATTGATAGTCCCTTGAACACACATGCTGCCGAGCT CTGGAAAAACCCACAGCTTTTAAGAAGTACCTGCAAGAAACCTACT CAAAGCAGGTCACCAGCCAGGAGCCCTGAGCGGCCGC