

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of UBE2R2

<u>Enzyme description:-</u>	UBE2R2
<u>Clone number:-</u>	DU4616
<u>Source:-</u>	BL21 recombinant
<u>Tag:-</u>	cleaved from N-terminal GST-tag
<u>Purification method:-</u>	GSH-Sepharose
<u>Expression level:-</u>	0.8mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	27560 Da
Average Mass	27576 Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	4.22
<u>Purity:-</u>	50%
<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

UBE2R2

<u>Protein</u>	UBE2R2
<u>Synonyms</u>	Ubc3B, Cdc34B
<u>Clone Number</u>	DU4616
<u>Species</u>	Human
<u>Accession Number</u>	Protein: Q712K3 DNA: NM_017811
<u>Tags</u>	N-terminal GST-tag (cleaved)
Aminoacid sequence of the purified protein	<u>GPLGSM</u><u>AQQQ</u><u>MTSSQ</u><u>KALMLEL</u><u>KS</u><u>LQ</u><u>EEP</u><u>VEG</u><u>FRIT</u><u>LV</u><u>DESD</u><u>LYN</u><u>WE</u><u>VA</u><u>I</u><u>F</u><u>G</u> <u>PPNT</u><u>LY</u><u>E</u><u>G</u><u>G</u><u>Y</u><u>F</u><u>K</u><u>A</u><u>H</u><u>I</u><u>K</u><u>F</u><u>P</u><u>I</u><u>D</u><u>P</u><u>Y</u><u>S</u><u>P</u><u>T</u><u>F</u><u>R</u><u>F</u><u>L</u><u>T</u><u>K</u><u>M</u><u>W</u><u>H</u><u>P</u><u>N</u><u>I</u><u>Y</u><u>E</u><u>N</u><u>G</u><u>D</u><u>V</u><u>C</u><u>I</u><u>S</u><u>I</u><u>L</u><u>H</u> <u>P</u><u>V</u><u>D</u><u>D</u><u>P</u><u>Q</u><u>S</u><u>G</u><u>E</u><u>L</u><u>P</u><u>S</u><u>E</u><u>R</u><u>W</u><u>N</u><u>P</u><u>T</u><u>Q</u><u>N</u><u>V</u><u>R</u><u>T</u><u>I</u><u>L</u><u>S</u><u>V</u><u>I</u><u>S</u><u>L</u><u>L</u><u>N</u><u>E</u><u>P</u><u>N</u><u>T</u><u>F</u><u>S</u><u>P</u><u>A</u><u>N</u><u>V</u><u>D</u><u>A</u><u>S</u><u>V</u><u>M</u><u>F</u><u>R</u><u>K</u><u>W</u> <u>R</u><u>D</u><u>S</u><u>K</u><u>G</u><u>K</u><u>D</u><u>K</u><u>E</u><u>Y</u><u>A</u><u>E</u><u>I</u><u>I</u><u>R</u><u>K</u><u>Q</u><u>V</u><u>S</u><u>A</u><u>T</u><u>K</u><u>A</u><u>E</u><u>A</u><u>E</u><u>K</u><u>D</u><u>G</u><u>V</u><u>K</u><u>V</u><u>P</u><u>T</u><u>T</u><u>L</u><u>A</u><u>E</u><u>Y</u><u>C</u><u>I</u><u>K</u><u>T</u><u>K</u><u>V</u><u>P</u><u>S</u><u>N</u><u>D</u><u>N</u><u>S</u> <u>D</u><u>L</u><u>L</u><u>Y</u><u>D</u><u>D</u><u>L</u><u>Y</u><u>D</u><u>D</u><u>D</u><u>I</u><u>D</u><u>D</u><u>E</u><u>D</u><u>E</u><u>E</u><u>E</u><u>E</u><u>D</u><u>A</u><u>D</u><u>C</u><u>Y</u><u>D</u><u>D</u><u>D</u><u>S</u><u>G</u><u>N</u><u>E</u><u>S</u>
Native sequence	in bold
Protease cleavage	Prescission site underlined
Cloning sites	BamH1 / NotI

DNA sequence of the expression cassette

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTCGAACCCAC
TCGACTTCTTTTGGAAATATCTTGAAGAAAAATATGAAGAGCATTTGTATG
AGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGTTTG
GAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAACACA
GTCTATGGCCATCATACGTTATATAGCTGACAAGCACAACATGTTGGGTG
GTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTG
GATATTAGATACGGTGTTCGAGAATTGCATATAGTAAAGACTTTGAAAC
TCTCAAAGTTGATTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTTCG
AAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCATGTAACCCAT
CCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATACATGGACCC
AATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAAAACGTATTG
AAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGCAAGTATATAGCA
TGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCC
AAAATCGGATCTGGAAGTTCTGTTCCAGGGGCCCCCTGGGATCCATGGCCC
AGCAGCAGATGACCAGCTCGCAGAAGGCCCTGATGCTCGAGCTGAAATCC
CTGCAGGAGGAACCGGTGGAGGGCTTCCGGATCACCTGGTGGACGAGTC
CGACCTCTACAACCTGGGAGGTGGCCATCTTCGGACCCCCCAACACCCTCT
ACGAAGGCGGCTACTTCAAGGCGCATATTAAATTTCTTATTGACTACCCC
TATTCACCACCTACCTTCAGATTCTTGACCAAAATGTGGCACCCCAACAT
TTATGAGAATGGAGATGTATGCATTTTCGATTCTTCATCCGCCTGTAGATG
ACCCACAGAGTGGAGAAGTGCCTTCTGAAAGGTGGAATCCTACTCAGAAT
GTGAGGACTATCCTATTAAGTGAATCTCACTGCTTAATGAGCCCAACAC
CTTCTCCCCAGCCAATGTCGATGCTTCAGTTATGTTTCAGGAAATGGAGAG
ACAGTAAAGGAAAAGACAAAGAATATGCTGAAATTATTAGGAAACAAGTT

TCAGCCACTAAGGCCGAAGCAGAGAAGGATGGAGTGAAGGTCCCCACAAC
CCTGGCGGAATACTGCATCAAACTAAAGTGCCTTCCAATGACAACAGCT
CAGATTTGCTTTACGACGACTTGTATGATGACGACATTGATGATGAAGAT
GAGGAGGAGGAAGATGCCGACTGTTATGATGATGATGATTCTGGGAATGA
GGAGTCGTGAGCGGCCGC