

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-UBE2K

<u>Enzyme description:-</u>	His-UBE2K
<u>Clone number:-</u>	DU20018
<u>Source:-</u>	BL21 recombinant
<u>Tag:-</u>	N-terminal His ₆ -tag
<u>Purification method:-</u>	Ni ⁺⁺ -NTA-Sepharose
<u>Expression level:-</u>	20mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	24835 Da
Average Mass	24850Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	6.17
<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-UBE2K

<u>Protein</u>	UBE2K
<u>Synonyms</u>	Ubc1, E2-25K, HIP2, HYPG, LIG
<u>Clone Number</u>	DU20018
<u>Species</u>	Human
<u>Accession Number</u>	Protein: NP_005330 DNA: NM_005339
<u>Tags</u>	N-terminal His ₆ tag
Aminoacid sequence of the expressed protein	MGSSHHHHHHSSG <u>LEVL</u> FQGP <u>GS</u> MANIAVQRIKREFKEVLKSEETSKNQIKV DLVDENFTEL <u>RGEI</u> AGPPDTPYEGGRYQLEIKIPETYFPNPPKVRFITKIWH PNISSVTGAI <u>CLDIL</u> KDQWAAAMTLRTVLLSLQALLAAAEPDDPQDAVVANQ YKQNP <u>EMFKQ</u> TARLWAHVYAGAPVSSPEYTKKIENLCAMGFDRNAVIVALSS KSWDVETATELLLSN
Native sequence	in bold
Protease cleavage	Prescission site underlined
Cloning sites	BamH1 / NotI

DNA sequence of insert

GGATCCATGGCCAACATCGCGGTGCAGCGAATCAAGCGGGAGTTCAAGGA
GGTGCCTGAAGAGCGAGGAGACGAGCAAAAATCAAATTAAGTAGATCTTG
TAGATGAGAATTTTACAGAATTAAGAGGAGAAATAGCAGGACCTCCAGAC
ACACCATATGAAGGAGGAAGATACCAACTAGAGATAAAAATACCAGAAAC
ATACCCATTTAATCCCCCTAAGGTCCGGTTTATCACTAAAATATGGCATC
CTAATATTAGTTCCGTACAGGGGCTATTTGTTTGGATATCCTGAAAGAT
CAATGGGCAGCTGCAATGACTCTCCGCACGGTATTATTGTCAATTGCAAGC
ACTATTGGCAGCTGCAGAGCCAGATGATCCACAGGATGCTGTAGTAGCAA
ATCAGTACAAACAAAATCCCGAAATGTTCAAACAGACAGCTCGACTTTGG
GCACATGTGTATGCTGGAGCACCAGTTTCTAGTCCAGAATACACCAAAAA
AATAGAAAACCTATGTGCTATGGGCTTTGATAGGAATGCAGTAATAGTGG
CCTTGTCTTCAAATCATGGGATGTAGAGACTGCAACAGAATTGCTTCTG
AGTAACTGAGCGGCCGC