

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of UBE2K

<u>Enzyme description:-</u>	UBE2K
<u>Clone number:-</u>	DU20018
<u>Source:-</u>	BL21 recombinant
<u>Tag:-</u>	cleaved from N-terminal His ₆ -tag
<u>Purification method:-</u>	Ni ⁺⁺ -NTA-Sepharose, protease treatment
<u>Expression level:-</u>	15mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	22690 Da
Average Mass	22703Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	5.29
<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

UBE2K

Protein UBE2K
Synonyms Ubc1, E2-25K, HIP2, HYPG, LIG
Clone Number DU20018
Species Human
Accession Number Protein: NP_005330 DNA: NM_005339

Tags N-terminal His₆ tag (cleaved)

Aminoacid sequence of the expressed protein **GPGSMANIAVQRIKREFKEVLKSEETSKNQIKVDLVDENFTELRG**E**IA**G**PPDTPYEGGRY**Q**LEIKIPETYPFNPPK**V**RFITKI**W**HPN**I**SSVT**G**AICLDILK**D**Q**W**
AAMTLRTVLLSL**Q**ALLAAAE**P**DD**P**QDAVVAN**Q**Y**K**Q**N**PE**M**FK**Q**TARL**W**AHVY**A**GA**P**VSSPEY**T**KK**I**ENLCAMGFDRNAV**I**VALSSKS**W**DVETATE**L**LS**N****

Native sequence in bold

Protease cleavage Precission site underlined

Cloning sites BamH1 / Not1

DNA sequence of insert **GGATCC**ATGGCCAACATCGCGGTGCAGCGAATCAAGCGGGAGTTCAAGGA
GGTGCTGAAGAGCGAGGAGACGAGCAAAAATCAAATTAAGTAGATCTTG
TAGATGAGAATTTTACAGAATTAAGAGGAGAAATAGCAGGACCTCCAGAC
ACACCATATGAAGGAGGAAGATACCAACTAGAGATAAAAATACCAGAAAC
ATACCCATTTAATCCCCCTAAGGTCCGGTTTATCACTAAAATATGGC**A**TC
CTAATATTAGTTCCGT**C**CAGGGGCTATTTGTTTGGATATCCTGAAAGAT
CAATGGGCAGCTGCAATGACTCTCCGCACGGTATTATTGT**C**ATTGCAAGC
ACTATTGGCAGCTGCAGAGCCAGATGATCCACAGGATGCTGTAGTAGCAA
ATCAGTACAAACAAAATCCCGAAATGTTCAAACAGACAGCTCGACTTTGG
GCACATGTGTATGCTGGAGCACCAGTTTCTAGTCCAGAATACACCAAAAA
AATAGAAAACCTATGTGCTATGGGCTTTGATAGGAATGCAGTAATAGTGG
CCTTGTCTTCAAATCATGGGATGTAGAGACTGCAACAGAATTGCTTCTG
AGTAACTGAGCGGCCGC