

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-UBE2K

<u>Enzyme description:-</u>	GST-UBE2K
<u>Clone number:-</u>	DU20013
<u>Source:-</u>	BL21 recombinant
<u>Tag:-</u>	N-terminal GST-tag
<u>Purification method:-</u>	GSH-Sepharose
<u>Expression level:-</u>	10mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	49199 Da
Average Mass	49229Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	5.98
<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

GST-UBE2K

Protein UBE2K
Synonyms Ubc1, E2-25K, HIP2, HYPG, LIG
Clone Number DU20013
Species Human
Accession Number Protein: NP_005330 DNA: NM_005339
Tags N-terminal GST-tag

Aminoacid sequence of the expressed protein
MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEF
PNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGAVLDIRY
GVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDHVTHPDFMLY
DALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIAWPLQGWQA
TFGGDHPPKSDLEVLFOGPLGSMANIAVQRIKREFKEVLKSEETSKNQIKV
DLVDENFTELARGEIAGPPDTPYEGGRYQLEIKIPETYPFNPPKVRFITKIWH
PNISSVTGAICLDILKDQWAAAMTLRTVLLSLQALLAAAEPDDPQDAVVANQ
YKQNPENFKQATARLWAHVYAGAPVSSPEYTKKIENLCAMGFDRNAVIVALSS
KSWDVETATELLLSN

Native sequence in bold
Protease cleavage Precission site underlined
Cloning sites BamH1 / NotI

DNA sequence of insert
GGATCCATGGCCAACATCGCGGTGCAGCGAATCAAGCGGGAGTTC AAGGA
GGTGCTGAAGAGCGAGGAGACGAGCAAAAATCAAATTAAGTAGATCTTG
TAGATGAGAATTTTACAGAATTAAGAGGAGAAATAGCAGGACCTCCAGAC
ACACCATATGAAGGAGGAAGATACCAACTAGAGATAAAAATACCAGAAAC
ATACCCATTTAATCCCCCTAAGGTCCGGTTTATCACTAAAATATGGCATC
CTAATATTAGTTCCTGCACAGGGGCTATTTGTTTGGATATCCTGAAAGAT
CAATGGGCAGCTGCAATGACTCTCCGCACGGTATTATTGTTCATTGCAAGC
ACTATTGGCAGCTGCAGAGCCAGATGATCCACAGGATGCTGTAGTAGCAA
ATCAGTACAAACAAAATCCCGAAATGTTCAAACAGACAGCTCGACTTTGG
GCACATGTGTATGCTGGAGCACCAGTTTCTAGTCCAGAATACACCAAAAA
AATAGAAAACCTATGTGCTATGGGCTTTGATAGGAATGCAGTAATAGTGG
CCTTGTCTTCAAATCATGGGATGTAGAGACTGCAACAGAATTGCTTCTG
AGTAACTGAGCGGCCG