

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-UBE2H

<u>Enzyme description:-</u>	GST-UBE2H
<u>Clone number:-</u>	DU4161
<u>Source:-</u>	BL21 recombinant
<u>Tag:-</u>	N-terminal GST-tag
<u>Purification method:-</u>	GSH-Sepharose
<u>Expression level:-</u>	3mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	47317 Da
Average Mass	47347 Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	4.94
<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-UBE2H

Protein UBE2H
Synonyms Ubc8, E2-20K, UbcH, UbcH2
Clone Number DU4161
Species Human
Accession Number Protein: NP_003335, DNA: NM_003344
Tags N-terminal GST-tag

Aminoacid sequence of the expressed protein **MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFOGPLGSSSPSPGKRMDTDVVKLIE**
SKHEVTILGGLNEFVVKFYGPQGTPEGGVWVVRVDLPDKYPFKSPSIGFMNKIFHPNIDEASGTVCLDVINQWTALYDLTNIFESFLPQLLAYPNPIDPLNGDAAAMYLHRPEEYKQKIKEYIQKYATEEALKEQEEGTGDSSSESSMSDFSEDEAQDMEL

Native sequence in bold, Start Met is missing
Protease cleavage Precission Protease site underlined
Cloning sites BamH1 / NotI

DNA sequence of the expression cassette ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGCCTTGTGCAACCCAC
TCGACTTCTTTTGAATATCTTGAAGAAAATATGAAGAGCATTTGTATG
AGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGTTTG
GAGTTTCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAACACA
GTCTATGGCCATCATACTTATATAGCTGACAAGCACAACATGTTGGGTG
GTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGTTTTG
GATATTAGATACGGTGTTCGAGAATTGCATATAGTAAAGACTTTGAAAC
TCTCAAAGTTGATTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTTCG
AAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCATGTAACCCAT
CCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATACATGGACCC
AATGTGCCTGGATGCGTTCCCAAATTAGTTTGTTTTAAAAACGTATTG
AAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGCAAGTATATAGCA
TGGCCTTTCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCC
AAAATCGGATCTGGAAGTTCTGTTCAGGGGCCCTGGGATCCTCATCTC
CCAGTCCGGGCAAGAGGCGGATGGACACGGACGTGGTCAAGCTCATCGAG
AGTAAACATGAGGTTACGATCCTGGGAGGACTTAATGAATTTGTAGTGAA
GTTTTATGGACCACAAGGAACACCATATGAAGGCGGAGTATGGAAAGTTA
GAGTGGACCTACCTGATAAATAACCCTTCAAATCTCCATCTATAGGATTC
ATGAATAAAATTTCCATCCCAACATTGATGAAGCGTCAGGAACGTGTGTG
TCTAGATGTAATTAATCAAACCTGGACAGCTCTCTATGATCTTACCAATA

TATTTGAGTCCTTCCTGCCTCAGTTATTGGCCTATCCTAACCCCATAGAT
CCTCTCAATGGTGACGCTGCAGCCATGTACCTCCACCGACCAGAAGAATA
CAAGCAGAAAATTAAAGAGTACATCCAGAAATACGCCACGGAGGAGGCGC
TGAAAGAACAGGAAGAGGGTACCGGGACAGCTCATCGGAGAGCTCTATG
TCTGACTTTTCCGAAGATGAGGCCAGGATATGGAGTTGTAGGCGGCCGC