

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-UBE2O

<u>Enzyme description:-</u>	His-UBE2O
<u>Clone number:-</u>	DU32152
<u>Source:-</u>	BL21 recombinant
<u>Tag:-</u>	N-terminal His ₆ -tag
<u>Purification method:-</u>	Ni ⁺⁺ -NTA-Sepharose
<u>Expression level:-</u>	0.1mg/L - frequent expression failures -
<u>Calculated molecular mass:-</u>	
Monoisotopic	84110 Da
Average Mass	84160 Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	4.68
<u>Purity:-</u>	40-70%
<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-UBE2O

Protein UBE2O
Synonyms E2-230K
Clone Number DU32152
Species Human
Accession Number Protein: Q9C0C9
Tags N-terminal His₆ tag

Aminoacid sequence of
the expressed protein

MGSSHHHHHHSSGLEVLFGPGSMTSADVMWQDGSVECNIRSNDLFPVHH
LDNNEFCPGDFVVDKRVQSCPDPVAVYGVVQSGDHIGRTC MVKWFKLRPSG
DDVELIGEEEDVSVYDIADHPDFRFRITDIVIRIGNTEDGAPHKEDEPSV
GQVARVDVSSKVEVWADNSKTIILPQHLYNIESEIEESDYDSVEGSTSG
ASSDEWEDSDSWETDNGLVEDEHPKIEEPPIPPLEQPVAPEDKGVVISE
EAATAAVQGA VAMAAPMAGLMEKAGKDGPPKSFRELKEAIKILES LKNMT
VEQLLTGSPTSPTVEPEKPTREKKFLDDIKKLOENLKKTLDNVAIVEEEK
MEAVPDVERKEDKPEGQSPVKA EWPSETPVLCQQCGGKPGVTFTSAKGEV
FSVLEFAPSNHSFKKIEFQPPEAKKFFSTVRKEMALLATSLPEGIMVKTF
EDRMDLFSALIKGPTRTPYEDGLYLFDIQLPNIYPVPPHFCYLSQCSGR
LNPPLYDNGKVCVSL LGTWIGKGTERTWTSKSSLLQVLISIQGLILVNEPY
YNEAGFDSDRGLQEGYENSRCYNEMALIRVVQSM TQLVRRPPEVFQEIR
QHFSTGGWRLVNRIESWLETHALLEKAQALPNGV PKASSPEPPAVAELS
DSGQQEPEDGGPAPGEASQGS DSEGGAQGLASASRDHTDQTSETAPDASV
PPSVKPKRRRKSYSRFLPEKSGYPDIGFPLFPLSKGFIKSIRGVLTOFRA
ALLEAGMPECTEDK

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

**DNA sequence of
insert**

GGATCCATGACCTCAGCCGACGTGATGTGGCAGGATGGCTCCGTGGAATG
CAACATCCGCTCCAACGACCTCTCCCTGTGCACCACCTGGACAACAACG
AGTTCCTGCCCTGGAGACTTCGTGGTAGATAAGCGAGTCCAGAGCTGTCCA
GACCTGCTGTCTACGGTGTGGTACAGTCTGGGGACCACATCGGCCGTAC
CTGCATGGTGAAGTGGTTCAAGCTGAGGCCGAGTGGGGACGACGTGGAGC
TGATTGGAGAAGAGGAAGATGTGAGTGTTTACGACATTGCTGACCACCCT
GACTTTAGGTTCCGTACA ACTGACATCGTCATCCGCATCGGCAACTACTGA
GGATGGGGCTCCTCACAAGGAGGATGAGCCATCGGTGGGCCAGGTGGCCC
GTGTGGACGTCAGCAGCAAGGTGGAGGTGGTGTGGGCTGACA ACTCAAAG
ACCATCATCTGCCCCAGCACTTGTACAACATAGAGTCTGAGATTGAGGA
GTCAGACTACGATTCGGTAGAAGGCAGCACCAGCGGGGCATCTCGGATG
AATGGGAAGATGATAGTGACAGCTGGGAGACGGACAATGGGCTGGTGGAG

GACGAGCACCCCAAGATAGAGGAGCCCCCATCCCACCCCTGGAGCAGCC
GGTGGCCCCTGAGGACAAGGGAGTGGTGATCAGTGAAGAGGCAGCCACAG
CTGCCGTCCAGGGGGCTGTGGCCATGGCTGCCCCATGGCCGGGCTGATG
GAGAAGGCTGGCAAGGACGGGCCACCCAAGAGCTTCCGGGAGTTGAAAGA
GGCCATCAAGATCCTGGAGAGCCTCAAGAACATGACTGTGGAGCAGCTGC
TGACGGGCTCGCCACCTCTCCGACTGTGGAGCCTGAGAAGCCAACCTCGG
GAGAAGAAGTTTCTGGATGACATCAAGAAGCTACAGGAAAACCTCAAGAA
GACCCCTGGACAATGTGGCCATTGTAGAGGAGGAGAAGATGGAAGCAGTGC
CCGACGTAGAGCGCAAGGAGGACAAGCCCGAGGGGCGAGTCACCTGTGAAG
GCTGAGTGGCCCAGCGAAACCCCGGTGCTGTGCCAGCAGTGTGGCGGCAA
GCCTGGCGTCACCTTCACCAGCGCCAAGGGCGAGGTCTTCTCCGTACTGG
AGTTTGCACCCTCAAATCATTCTTTTAAGAAAATTGAGTTCCAGCCTCCA
GAAGCCAAGAAGTTCTTCAGCACAGTGCGGAAGGAGATGGCGCTGCTGGC
TACCTCACTGCCTGAGGGCATCATGGTCAAGACTTTTGAAGATAGAATGG
ACCTCTTCTCAGCTCTCATCAAGGGCCCCACTCGAACCCCTACGAGGAT
GGCCTCTACTTGTGTGACATCCAGCTCCCCAACATCTACCCAGCCGTGCC
CCCCACTTCTGCTACCTCTCCCAATGCAGTGGCCGCCTGAACCCCAACC
TGTATGACAATGGGAAGGTGTGTGTCAGCCTCCTGGGCACCTGGATTGGA
AAGGGGACAGAGAGGTGGACAAGCAAGTCCAGCCTTCTCCAGGTGCTCAT
CTCCATCCAAGGTCTGATCCTGGTAAATGAACCATACTACAACGAAGCCG
GCTTCGACAGTGACCGAGGCCTGCAGGAAGGCTATGAAAACAGTCGCTGT
TACAATGAGATGGCGCTGATCCGCGTGGTGCAGTCCATGACCCAGCTGGT
GCGGCGGCCCCCCGAGGTCTTTGAGCAGGAGATCAGGCAACACTTTAGCA
CTGGTGGCTGGCGGCTGGTGAACCGTATCGAGTCCTGGCTGGAAACCCAT
GCCCTGCTGGAGAAGGCCCAGGCACTGCCCAACGGGGTGCCCAAGGCCAG
CAGCTCGCCAGAGCCCCCAGCTGTAGCCGAGCTGTCAGACTCCGGCCAAC
AAGAACCTGAGGATGGAGGGCCAGCCCCAGGAGAGGCCTCCAGGGCTCA
GACTCAGAGGGCGGTGCCAGGGCCTGGCCTCAGCTAGCAGGGACCACAC
AGACCAGACTTCGGAGACCGCACCAGACGCATCGGTGCCACCCAGTGTGA
AACCAAAGAAGCGGAGAAAGAGCTACCGGAGCTTCTTACCTGAGAAGAGT
GGCTACCCTGACATCGGCTTCCCCCTTCCCCTTTTCCAAGGGTTTTTCA
CAAGAGCATCCGGGGTGTCTGACGCAGTTCCGGGCTGCCCTGCTAGAGG
CAGGCATGCCGGAGTGCACAGAGGACAAGTAGGCGGCCGC