

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-UBE1

<u>Enzyme description:-</u>	UBE1
<u>Clone number:-</u>	DU32888
<u>Source:-</u>	Sf21 recombinant
<u>Tag:-</u>	His-
<u>Purification method:-</u>	Ni ⁺⁺ -Sepharose
<u>Expression level:-</u>	7mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	121012 Da
Average Mass	121084 Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	5.1
<u>Purity:-</u>	90%
<u>Enzyme storage buffer:-</u>	
50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT	
<u>Storage temperature:-</u>	-80°C
<u>Assay:-</u>	

Any E2-loading assay or E3 assay; e.g. UBE2N/UBE2V1 K63 chain formation.

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Clone Data Sheet

His-UBE1

Protein UBE1
Clone Number DU32888
Species Human
Accession Number P22314
Tags His₆-

Aminoacid sequence of
the expressed protein

MSYYHHHHHDYDIP^TTENLYFQGAMGSSSSPLSKRRVSGPDPKPGSNC
SPAQSVLSEVPSVPTNGMAKNGSEADIDEGLYSRQLYVLGHEAMKRLQTS
SVLVSGLRGLGVEIAKNIILGGVKAVTLHDQGTAWADLSSQFYLRREEDI
GKNRAEVSQPRLAELNSYVPVTAYTGPLVEDFLSGFQVVVLTNTPLEDQL
RVGEFCHNRGIKLVVADTRGLFGQLFCDFGEEMILTDSNGEQPLSAMVSM
VTKDNPGVVTCLDEARHGFESGDFVSFSEVQGMVELNGNQPMKIKVLGPY
TFSICDTSNFSYIRGGIVSQVKVPKKISFKSLVASLAEPDFVVTDFAKF
SRPAQLHIGFQALHQFCAQHGRPPRPRNEEDAAELVALAQAVNARALPAV
QQNNLDEDLIRKLAYVAAGDLAPINAFIGGLAAQEVKACSGKFMPIQW
LYFDALECLPEDKEVLTEDEKCLQRQNRDYGQVAVFGSDLOEKLGKQKYFL
VGAGAIGCELLKNFAMIGLGCGEIIVTDMDTIEKSNLNRQFLFRPWD
VTKLKSDTAAAVRQMNPHIRVTSHQNRVGPDTERIYDDDFQNLGQVAN
ALDNVDARMYMDRRCVYRKPLLESGLTGTGKGNVQVVIPLTESYSSSQD
PPEKSIPICTLKNFPNAIEHTLQWARDEFEGFLFKQPAENVNQYLTPKFV
ERTLRLAGTQPLEVLEAVQRSVLQRPQTWADCVTWACHHWHTQYSNNIR
QLLHNFPPDQLTSSGAPFWSGPKRCPHPLTFDVNNPLHLDYVMAAANLFA
QTYGLTGSQDRAAVATFLQSVQVPEFTPKSGVKIHVSDQELQSANASVDD
SRLEELKATLPSDKLPGFKMYPIDFEKDDSNFHMDFIVAASNRAENY
DIPSAHRHKSCLIAGKIIPAIATTTAAVVGLVCELYKVVQGHRQLDSYK
NGFLNLALPFFGFSEPLAAPRHQYYNQEWTLWDRFEVQGLQPNGEEMTLK
QFLDYFKTEHKLEITMLSQGVSMYSFFMPAAKLERLDQPMTEIVSRVS
KRKLGRHVRALVLELCCNDESGEDVEVPYVRYTIR

Native sequence

Protease cleavage TEV underlined

Cloning sites BamHI

**DNA sequence of
insert**

GGATCCTCCAGCTCGCCGCTGTCCAAGAAACGTCGCGTGTCCGGGCCTGA
TCCAAAGCCGGGTCTAACTGCTCCCCTGCCAGTCCGTGTTGTCCGAAG
TGCCCTCGGTGCCAACCAACGGAATGGCCAAGAACGGCAGTGAAGCAGAC
ATAGACGAGGGCCTTTACTCCCGGCAGCTGTATGTGTTGGGCCATGAGGC
AATGAAGCGGCTCCAGACATCCAGTGTCTGGTATCAGGCCTGCGGGGCC
TGGGCGTGGAGATCGCTAAGAACATCATCCTTGGTGGGGTCAAGGCTGTT
ACCCTACATGACCAGGGCACTGCCAGTGGGCTGATCTTTCCCTCCAGTT
CTACCTGCGGGAGGAGGACATCGGTAAAAACCGGGCCGAGGTATCACAGC
CCCGCCTCGCTGAGCTCAACAGCTATGTGCCTGTCACTGCCTACACTGGA
CCCCCTCGTTGAGGACTTCCTTAGTGGTTTCCAGGTGGTGGTGTCCACAA
CACCCCCCTGGAGGACCAGCTGCGAGTGGGTGAGTTCTGTACAAACCGTG
GCATCAAGCTGGTGGTGGCAGACACGCGGGGCCCTGTTTGGGCAGCTCTTC
TGTGACTTTGGAGAGGAAATGATCCTCACAGATTCCAATGGGGAGCAGCC
ACTCAGTGTCTATGGTTTCTATGGTTACCAAGGACAACCCCGGTGTGGTTA
CCTGCCTGGATGAGGCCCGACACGGGTTTGGAGAGCGGGGACTTTGTCTCC
TTTTTCAGAAGTACAGGGCATGGTTGAACTCAACGGAAATCAGCCCATGGA
GATCAAAGTCTGGGTCTTATACCTTTAGCATCTGTGACACCTCCAACCT
TCTCCGACTACATCCGTGGAGGCATCGTCAGTCAGGTCAAAGTACCTAAG
AAGATTAGCTTTAAATCCTTGGTGGCCTCACTGGCAGAACCTGACTTTGT
GGTGACGGACTTCGCCAAGTTTTCTCGCCCTGCCAGCTGCACATTGGCT
TCCAGGCCCTGCACCAGTTCTGTGCTCAGCATGGCCGGCCACCTCGGCC
CGCAATGAGGAGGATGCAGCAGAACTGGTAGCCTTAGCACAGGCTGTGAA
TGCTCGAGCCCTGCCAGCAGTGCAGCAAATAACCTGGACGAGGACCTCA
TCCGGAAGCTGGCATAATGTGGCTGCTGGGGATCTGGCACCCATAAACGCC
TTCATTGGGGCCCTGGCTGCCAGGAAGTCATGAAGGCCTGCTCCGGGAA
GTTTATGCCCATCATGCAGTGGCTATACTTTGATGCCCTTGAGTGTCTCC
CTGAGGACAAAGAGGTCCTCACAGAGGACAAGTGCCTCCAGCGCCAGAAC
CGTTATGACGGGCAAGTGGCTGTGTTTGGCTCAGACCTGCAAGAGAAGCT
GGGCAAGCAGAAGTATTTCTGGTGGGTGCGGGGCCATTGGCTGTGAGC
TGCTCAAGAACTTTGCCATGATTGGGCTGGGCTGCGGGGAGGGTGGAGAA
ATCATCGTTACAGACATGGACACCATTGAGAAGTCAAATCTGAATCGACA
GTTTCTTTTCCGGCCCTGGGATGTCACGAAGTTAAAGTCTGACACGGCTG
CTGCAGCTGTGCGCCAAATGAATCCACATATCCGGGTGACAAGCCACCAG
AACCGTGTGGGTCTTGACACGGAGCGCATCTATGATGACGATTTTTTTCCA
AAACCTAGATGGCGTGGCCAATGCCCTGGACAACGTGGATGCCCGCATGT
ACATGGACCGCCGCTGTGTCTACTACCGGAAGCCACTGCTGGAGTCAGGC
ACACTGGGCACCAAGGGCAATGTGCAGGTGGTGTATCCCTTCTTGACAGA
GTCGTACAGTTCCAGCCAGGACCCACCTGAGAAGTCCATCCCCATCTGTA
CCCTGAAGAACTTCCCTAATGCCATCGAGCACACCCTGCAGTGGGCTCGG
GATGAGTTTGAAGGCCTCTTCAAGCAGCCAGCAGAAAATGTCAACCAGTA
CCTCACAGACCCCAAGTTTGTGGAGCGAACACTGCGGCTGGCAGGCACTC
AGCCCTTGGAGGTGCTGGAGGCTGTGCAGCGCAGCCTGGTGTGTCAGCGA
CCACAGACCTGGGCTGACTGCGTGACCTGGGCCTGCCACCCTGGCACAC
CCAGTACTCGAACAAACATCCGGCAGCTGCTGCACAACCTTCCCTCCTGACC
AGCTCACAAGCTCAGGAGCGCCGTTCTGGTCTGGGCCCAAACGCTGTCCA
CACCCGCTCACCTTTGATGTCAACAATCCCCTGCATCTGGACTATGTGAT
GGCTGCTGCCAACCTGTTTGGCCAGACCTACGGGCTGACAGGCTCTCAGG
ACCGAGCTGCTGTGGCCACATTCCTGCAGTCTGTGCAGGTCCCCGAATTC
ACCCCAAGTCTGGCGTCAAGATCCATGTTTCTGACCAGGAGCTGCAGAG
CGCCAATGCCTCTGTTGATGACAGTCTGTCTAGAGGAGCTCAAAGCCACTC
TGCCCAGCCCAGACAAGCTCCCTGGATTCAAGATGTACCCCATTGACTTT
GAGAAGGATGATGACAGCAACTTTCATATGGATTTCATCGTGGCTGCATC

CAACCTCCGGGCAGAAAAC TATGACATTCCTTCTGCAGACCGGCACAAGA
GCAAGCTGATTGCAGGGAAGATCATCCCAGCCATTGCCACGACCACAGCA
GCCGTGGTTGGCCTTGTGTGTCTGGAGCTGTACAAGGTTGTGCAGGGGCA
CCGACAGCTTGACTCCTACAAGAATGGTTTCCTCAACTTGGCCCTGCCTT
TCTTTGGTTTCTCTGAACCCCTTGCCGCACCACGTCACCAGTACTATAAC
CAAGAGTGGACATTGTGGGATCGCTTTGAGGTACAAGGGCTGCAGCCTAA
TGGTGAGGAGATGACCCTCAAACAGTTCCTCGACTATTTTAAGACAGAGC
ACAAATTAGAGATCACCATGCTGTCCCAGGGCGTGTCCATGCTCTATTCC
TTCTTCATGCCAGCTGCCAAGCTCAAGGAACGGTTGGATCAGCCGATGAC
AGAGATTGTGAGCCGTGTGTCGAAGCGAAAGCTGGGCCGCCACGTGCGGG
CGCTGGTGCTTGAGCTGTGCTGTAACGACGAGAGCGGGCAGGATGTCGAG
GTTCCCTATGTCCGATACACCATCCGCTGAGGATCC