

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of UBE1 [2 - 1058]

<u>Enzyme description:-</u>	UBE1 [2 – 1058]
<u>Clone number:-</u>	DU 3026
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6)
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose
<u>Expression level:-</u>	1 mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	120, 978.72 daltons
Average Mass	121, 055.42 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	5.55
<u>Purity:-</u>	85 %
<u>Enzyme storage buffer:-</u>	
50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.	
<u>Storage temperature:-</u>	-70 °C
<u>Assay:-</u>	Ubiquitin assay

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Clone Data Sheet

UBE1 [2 - 1058]

Protein UBE1 [2 - 1058]

Clone Number DU 3026

Species Human

Accession number X56976

Tags N-terminal His(6)

Bacterially expressed protein

MSYYHHHHHDYDIPTTENLYFQAMGSSSSPLSKRRVSGPDPKPGSN
CSPAQSVLSEVPSVPTNGMAKNGSEADIDEGLYSRQLYVLGHEAMKRLQ
TSSVLVSGLRGLGVEIAKNIILGGVKAVTLHDQGTAWADLSSQFYLRE
EDIGKNRAEVSQPRLAELNSYVPVTAYTGPLVEDFLSGFQVVVLTNTPL
EDQLRVGEI^ICHNRGIKLVVAD^{TR}GLFGQLFCDFGEEMILTDSNGEQPLS
AMVSMVTKDNPVVTCLEARHGFESGDFVSFSEVOGMVELNGNQPMI
KVLGPYTF^SICDTSNFS^{DY}IRGGIVS^{QV}KVP^{KK}ISFKSLVASLAEPDFV
VTDFAKFSRPAQLHIGFQALHQFCAQHGRPPRPRNEEDAAELVALAQAV
NARALPAVQONNLEDLIRKLAYVAAGDLAPINAFIGGLAAQEVKACS
GKFMPIMQWLYFDALCLPEDKEVLTE^{DK}CLQRQ^{RY}DGQ^{VA}VFGSD^{LQ}
EKLKQ^{KY}FLVGAGAI^GCELLKNFAMI^{GL}CGEGGEI^IVTDM^{DT}IEKSN
LNRQ^{FL}FRP^{VD}TKL^{KS}DTAA^{AA}VR^{OM}NPHIR^{VT}SHQ^{NR}VGP^{DT}ERI^{YD}
DDFFQ^{NLD}GVANALDNVDAR^{MY}DRRCV^{YR}KPLLES^{GT}LGT^{KG}NVQ^{VV}
IPFLTESYSSQDPPEKSIPICT^{LN}FPNAIEHTLQ^{WAR}DEFEG^{LF}KQ^P
AENVN^{OYL}TDPKFVERTLRLAG^{TQ}PLEVLEAV^{QR}SLV^{LQ}R^{PQ}TWADC^{VT}
WACHHWHTQ^{YS}NNIRQLL^{HN}FPPD^{QL}TSSGAP^{FW}SGPK^{RC}PHPLTF^{DVN}
NPLHLDYVMAAANLFAQ^{TY}GLTGSQ^{DR}AAVAT^{FL}QSV^{VQ}VEFT^{PK}SG^{VK}
IHVSDQELQSANASVDDSRLEELKATLPS^{PK}LPG^{FK}MPID^{FE}KDD^{DS}
NFHMD^{FI}VAA^{SN}LRAENYDIP^SADR^{HK}SKLIAGKIIPAIATTTAAV^{VGL}
VCLELYKVVQ^{GR}QLDSYKNG^{FL}NLALP^{FF}GFSEPLA^{AP}RHQ^YYNQ^{EW}T
LWDRFEVQ^{GL}QPN^{GE}MTL^{KQ}FLDYFKTE^{HK}LEIT^{ML}SQ^{GV}SML^{YS}FF^{FM}
PAAKLKERLDQ^{PM}TEIVSRVSK^{RK}LGRH^{VR}ALVLELCCN^{DES}GED^{VE}VP
YVRYTIR

Native sequence Amino acids S2 – R1058 (end) of UBE1.
Residue S29 of the fusion protein is equivalent to S2 of the native enzyme.
The His(6) tag is located at residues 5 – 10.

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The following amino acid substitutions are present:

F – I, where F178 of the native enzyme is I205 of the fusion protein

G – D, where G190 of the native enzyme is D217 of the fusion protein

Protease cleavage rTEV (ENLYFQG) residues 18 - 24

Cloning sites *Bam*H1 site of pFastBac HTb

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Nucleotide sequence of insert

ggatccTCCAGCTCGCCGCTGTCCAAGAAACGTCGCGTGTCCGGGCCTG
ATCCAAAGCCGGTTCTAACTGCTCCCCTGCCAGTCCGTGTTGTCCGA
AGTGCCCTCGGTGCCAACCAACGGAATGGCCAAGAACGGCAGTGAAGCA
GACATAGACGAGGGCCTTTACTCCCGGCAGCTGTATGTGTTGGGCCATG
AGGCAATGAAGCGGCTCCAGACATCCAGTGTCTTGGTATCAGGCCTGCG
GGCCTGGGCGTGGAGATCGCTAAGAACATCATCCTTGGTGGGGTCAAG
GCTGTTACCCTACATGACCAGGGCACTGCCAGTGGGCTGATCTTTTCT
CCCAGTTCTACCTGCGGGAGGAGGACATCGGTAAAAACGGGCCGAGGT
ATCACAGCCCCGCTCGCTGAGCTCAACAGCTATGTGCCTGTCACTGCC
TAACTGGACCCCTCGTTGAGGACTTCTTAGTGGTTTCCAGGTGGTGG
TGCTACCAACACCCCCCTGGAGGACCAGCTGCGAGTGGGTGAGATCTG
TCACAACCGTGGCATCAAGCTGGTGGTGGCAGACACGCGGGGCCTGTTT
GGCAGCTCTTCTGTGACTTTGGAGAGGAAATGATCCTCACAGATTCCA
ATGGGGAGCAGCCACTCAGTGCTATGGTTTCTATGGTTACCAAGGACAA
CCCCGGTGTGGTTACCTGCCTGGATGAGGCCCGACACGGGTTTGGAGAGC
GGGACTTTGTCTCCTTTTTCAGAAAGTACAGGGCATGGTTGAACTCAACG
GAAATCAGCCCATGGAGATCAAAGTCTGGGTCTTATACCTTTAGCAT
CTGTGACACCTCCAACTTCTCCGACTACATCCGTGGAGGCATCGTCAGT
CAGGTCAAAGTACCTAAGAAGATTAGCTTTAAATCCTTGGTGGCCTCAC
TGGCAGAACCTGACTTTTGTGGTGACGGACTTCGCCAAGTTTTCTCGCCC
TGCCCAGCTGCACATTGGCTTCCAGGCCCTGCACCAGTTCTGTGCTCAG
CATGGCCGGCCACCTCGGCCCGCAATGAGGAGGATGCAGCAGAACTGG
TAGCCTTAGCACAGGCTGTGAATGCTCGAGCCCTGCCAGCAGTGCAGCA
AAATAACCTGGACGAGGACCTCATCCGGAAGCTGGCATATGTGGCTGCT
GGGGATCTGGCACCCATAAACGCCTTTTCATTTGGGGCCCTGGCTGCCCAGG
AAGTCATGAAGGCCTGCTCCGGGAAGTTCATGCCATCATGCAGTGGCT
ATACTTTGATGCCCTTGAGTGTCTCCCTGAGGACAAAGAGGTCTCAC
GAGGACAAGTGCCCTCCAGCGCCAGAACCGTTATGACGGGCAAGTGGCTG
TGTTTGGCTCAGACCTGCAAGAGAAGCTGGGCAAGCAGAAGTATTTCT
GGTGGGTGCGGGGGCCATTGGCTGTGAGCTGCTCAAGAACTTTGCCATG
ATTGGCTGGCTGCGGGGAGGGTGGAGAAATCATCGTTACAGACATGG
ACACCATTGAGAAGTCAAATCTGAATCGACAGTTTCTTTTCCGGCCCTG
GGATGTCACGAAGTTAAAGTCTGACACGGCTGCTGCAGCTGTGCGCCAA
ATGAATCCACATATCCGGGTGACAAGCCACCAGAACCGTGTGGGTCTG
ACACGGAGCGCATCTATGATGACGATTTTTTTCCAAAACCTAGATGGCGT
GGCCAATGCCCTGGACAACGTGGATGCCCGCATGTACATGGACCGCCGC
TGTGTCTACTACCGGAAGCCACTGCTGGAGTCAGGCACACTGGGCACCA
AAGGCAATGTGCAGGTGGTGTATCCCCTTCTGACAGAGTCGTACAGTTC
CAGCCAGGACCCACCTGAGAAGTCCATCCCCATCTGTACCCTGAAGAAC
TTCCCTAATGCCATCGAGCACACCCTGCAGTGGGCTCGGGATGAGTTTG
AAGGCCTCTTCAAGCAGCCAGCAGAAAATGTCAACCAGTACCTCACAGA
CCCCAAGTTTGTGGAGCGAACACTGCGGCTGGCAGGCACTCAGCCCTTG
GAGGTGCTGGAGGCTGTGCAGCGCAGCCTGGTGTGCTGCAGCGACCACAGA
CCTGGGCTGACTGCGTGACCTGGGCCTGCCACCACTGGCACACCCAGTA
CTCGAACAACATCCGGCAGCTGCTGCACAACCTCCCTCCTGACCAGCTC
ACAAGCTCAGGAGCGCCGTTCTGGTCTGGGCCCAAACGCTGTCCACACC
CGCTCACCTTTGATGTCAACAATCCCCTGCATCTGGACTATGTGATGGC

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TGCTGCCAACCTGTTTGCCCAGACCTACGGGCTGACAGGCTCTCAGGAC
CGAGCTGCTGTGGCCACATTCCTGCAGTCTGTGCAGGTCCCCGAATTCA
CCCCAAGTCTGGCGTCAAGATCCATGTTTCTGACCAGGAGCTGCAGAG
CGCCAATGCCTCTGTTGATGACAGTCGTCTAGAGGAGCTCAAAGCCACT
CTGCCCAGCCCAGACAAGCTCCCTGGATTCAAGATGTACCCCATTTGACT
TTGAGAAGGATGATGACAGCAACTTTCATATGGATTTTCATCGTGGCTGC
ATCCAACCTCCGGGCAGAAAACCTATGACATTCCTTCTGCAGACCGGCAC
AAGAGCAAGCTGATTGCAGGGAAGATCATCCCAGCCATTGCCACGACCA
CAGCAGCCGTGGTTGGCCTTGTGTGTCTGGAGCTGTACAAGGTTGTGCA
GGGCACCGACAGCTTGACTCCTACAAGAATGGTTTTCTCAACTTGGCC
CTGCCTTTCCTTGGTTTTCTCTGAACCCCTTGCCGCACCACGTCACCAGT
ACTATAACCAAGAGTGGACATTGTGGGATCGCTTTGAGGTACAAGGGCT
GCAGCCTAATGGTGAGGAGATGACCCTCAAACAGTTCCTCGACTATTTT
AAGACAGAGCACAAATTAGAGATCACCATGCTGTCCCAGGGCGTGTCCA
TGCTCTATTCCTTCTTCATGCCAGCTGCCAAGCTCAAGGAACGGTTGGA
TCAGCCGATGACAGAGATTGTGAGCCGTGTGTCGAAGCGAAAGCTGGGC
CGCCACGTGCGGGCGCTGGTGCTTGAGCTGTGCTGTAACGACGAGAGCG
GCGAGGATGTCGAGGTTCCCTATGTCCGATACACCATCCGctgaggatc

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