

MRCPPU REAGENTS and SERVICES

Standard Operating Procedure

Preparation of UBE3A C843A [1 – 875]

Enzyme description:- UBE3A C843A [1 - 875]

Clone number:- DU 65473

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Agarose

Calculated molecular mass:-

Monoisotopic 127, 252.85 daltons

Average Mass 127, 334.43 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.21

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 0.5 mM TCEP

Storage temperature:- -70 °C

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Clone Data Sheet

UBE3A C843A [1 - 875]

Protein UBE3A C843A [1 - 875]

Clone number DU 65473

Species Human

Accession number Q05086-1

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA
VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDENLYFQGG**MEKLNHCYWKSGEPQS**
DDIEASRMKRAAAKHLIERYYHQLTEGCGNEACTNEFCASCPTFLRMDN
NAAAIKALELYKINAKLCDPHPSKKGASSAYLENSK GAPNNSCSEIKMN
KKGARIDFKDVTYLTEEKVYEIILELCREREDYSPLIRVIGRVFSSAEAL
VQSF RKVKQHTKEELKSLQAKDEDEKDEDEKEKAACSAAMEEDSEASSS
RIGDSSQGDNNLQKLGPDVSV DIDAIRRVYTRLLSNEKIETAFLNALV
YLS PNVECDLTYHNVYSRDPNYLNLFIIVMENRNLHSPEYLEMALPLFC
KAMSKLPLAAQGKLIRLWSKYNADQIRRMETFOQLITYKVISNEFNSR
NLVNDDDAIVAASKCLKMVYANVVGGEVDTNHNEEDDEEPIPESSLT
LQELLGEERNKKGPRVDPLETELGVKTLDCRKPLIPFEFFINEPLNEV
LEM DKDYTFKVE TENKFSFMTCPFILNAVTKNLGLYYDN RIRMY SERR
ITVLYSLVQGGQLNPYLRLKVR RDHIIDDALVRLEM IAMENPADLKKQL
YVEFEGEQGVDEGGVSKEFFQLVVEEIFNPDIGMFTYDESTKLFWFNPS
SFETEGQFTLIGIVLGLAIYNNCILDVHFPMVVYRKL MGKKGTFRDLGD
SHPVLYQSLKDLLEYEGNVEDDMMITFQISQTDLFGNPMMYDLKENGDK
IPITNENRKEFVNLYSDYILNKSVEKQFKAFRRGFHMVTNESPLKYLFR
PEEIELLICGSRNLDFQALEETTEYDGGYTRDSVLIREFWEIVHSFTDE
QKRLFLQFTTGTDRAPVGGGLGKLMIIAKNGP DTERLPTSHTA**FNVLLL**
PEYSSKEK LKERLLKAITYAKGFGML

Native sequence Amino acids M1 – L975 (end residue) of human UBE3A.
Residue M230 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

The enzyme has an C843**A** mutation. Residue C843 is equivalent to **A1072** of the fusion protein.

Protease cleavage TEV (**ENLYFQG**) residues 221 – 227

Cloning sites *Bam*H1 and *Not*1 sites of pGEX 6P-1

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Nucleotide Sequence

ggatccATGGAGAAGCTGCACCAGTGTATTATTGGAAATCAGGAGAACCTCAGTCTGACGA
CATTGAAGCTAGCCGAATGAAGCGAGCAGCTGCAAAGCATCTAATAGAACGCTACTACC
ACCAGTTAACTGAGGGCTGTGGAAATGAAGCCTGCACGAATGAGTTTTGTGCTTCCTGT
CCAACTTTTCTTCGTATGGATAATAATGCAGCAGCTATTAAAGCCCTCGAGCTTTATAA
GATTAATGCAAACTCTGTGATCCTCATCCCTCCAAGAAAGGAGCAAGCTCAGCTTACC
TTGAGAACTCGAAAGGTGCCCCCAACAACCTCTGCTCTGAGATAAAAAATGAACAAGAAA
GGCGCTAGAATTGATTTTTAAAGATGTGACTTACTTAACAGAAGAGAAGGTATATGAAAT
TCTTGAATTATGTAGAGAAAGAGAGGATTATCCCTTTAATCCGTGTTATTGGAAGAG
TTTTTTCTAGTGCTGAGGCATTGGTACAGAGCTTCCGGAAAGTTAAACAACACACCAAG
GAAGAACTGAAATCTCTTCAAGCAAAAGATGAAGACAAAGATGAAGATGAAAAGGAAAA
AGCTGCATGTTCTGCTGCTGCTATGGAAGAAGACTCAGAAGCATCTTCCTCAAGGATAG
GTGATAGCTCACAGGGAGACAACAATTTGCAAAAATTAGGCCCTGATGATGTGTCTGTG
GATATTGATGCCATTAGAAGGGTCTACACCAGATTGCTCTCTAATGAAAAAATTGAAAC
TGCTTTTCTCAATGCACTTGTATATTTGTCACCTAACGTGGAATGTGACTTGACGTATC
ACAATGTATACTCTCGAGATCCTAATTATCTGAATTTGTTCAATTATCGTAATGGAGAAT
AGAAATCTCCACAGTCCTGAATATCTGGAAATGGCTTTGCCATTATTTTGCAAAGCGAT
GAGCAAGCTACCCCTTGCAGCCCAAGGAAAACCTGATCAGACTGTGGTCTAAATACAATG
CAGACCAGATTCGGAGAATGATGGAGACATTTAGCAACTTATTACTTATAAAGTCATA
AGCAATGAATTTAACAGTCGAAATCTAGTGAATGATGATGATGCCATTGTTGCTGCTTC
GAAGTGCTTGAAAATGGTTTACTATGCAAATGTAGTGGGAGGGGAAGTGGACACAAATC
ACAATGAAGAAGATGATGAAGAGCCCATCCCTGAGTCCAGCGAGCTGACACTTCAGGAA
CTTTTGGGAGAAGAAAGAAGAAACAAGAAAGGTCCTCGAGTGGACCCCTGGAAACTGA
ACTTGGTGTAAAACCTGGATTGTGCAAAACCACTTATCCCTTTTGAAGAGTTTATTA
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TTTGGGATTATATTATGACAATAGAATTCGCATGTACAGTGAACGAAGAATCACTGTTT
TCTACAGCTTAGTTCAAGGACAGCAGTTGAATCCATATTTGAGACTCAAAGTTAGACGT
GACCATATCATAGATGATGCACTTGTCCGGCTAGAGATGATCGCTATGGAAAATCCTGC
AGACTTGAAGAAGCAGTTGTATGTGGAATTTGAAGGAGAACAAGGAGTTGATGAGGGAG
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ATGTTACATACGATGAATCTACAAAATTGTTTTGGTTAATCCATCTTCTTTTGAAC
TGAGGGTCAGTTTACTCTGATTGGCATAGTACTGGGTCTGGCTATTTACAATAACTGTA
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TTTTTGGTAACCCAATGATGTATGATCTAAAGGAAAATGGTGATAAAATTTCCAATTACA
AATGAAAACAGGAAGGAATTTGTCAATCTTTATTCTGACTACATTCTCAATAAATCAGT
AGAAAAACAGTTCAAGGCTTTTTCGGAGAGGTTTTTCATATGGTGACCAATGAATCTCCCT
TAAAGTAC TTATTTCAGACCAGAAGAAATTTGAATTGCTTATATGTGGAAGCCGGAATCT
AGATTTCCAAGCACTAGAAGAACTACAGAATATGACGGTGGCTATACCAGGGACTCTG
TTCTGATTAGGGAGTTCTGGGAAATCGTTTCATTTCATTTACAGATGAACAGAAAAGACTC
TTCTTGCAAGTTTACAACGGGCACAGACAGAGCACCTGTGGGAGGACTAGGAAAATTTAAA
GATGATTATAGCCAAAAATGGCCAGACACAGAAAGGTTACCTACATCTCATACTgcCT
TTAATGTGCTTTTACTTCCGGAATACTCAAGCAAAGAAAACTTAAAGAGAGATTGTTG
AAGGCCATCACGTATGCCAAAGGATTTGGCATGCTGTAAAgcgccgc