

MRCPPU REAGENTS and SERVICES

Standard Operating Procedure

Preparation of active UBE3A [518 – 875]

Enzyme description:- UBE3A [518 - 875]

Clone number:- DU 65569

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Agarose

Calculated molecular mass:-

Monoisotopic 68, 127.48 daltons

Average Mass 68, 171.44 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.27

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 [518 - 875]

<u>Protein</u>	UBE3A [518 - 875]
<u>Clone number</u>	DU 65569
<u>Species</u>	Human
<u>Accession number</u>	Q05086-1
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDENLYFQGGSQLNPYLRLKVRDHI I DDALVRLEMIAMENPADLKKQLYVEFEQEQQVDEGGVSKEFFQLVVEEI FNPDIGMFTYDESTKLFWFNPSSFETEGQFTLIGIVLGLAIYNNCILDV HFPMVVYRKL MGKGTFRDLGDSHPVLYQSLKDLLEYEGNVEDDMMITF QISQTDLEFGNPMYDLKENGDKIPITNENRKEFVNLYSDYILNKSVEKQ FKAFRRGFHMVTNESPLKYLFRPEEIELLICGSRNLDFQALEETTEYDG GYTRDSVLIREFWEIVHSFTDEQKRLFLQFTTGTDRAPVGGGLGKLKMI I AKNGPDTERLPTSHTCFNVLLLPEYSSKEKLKERLLKAITYAKGFGML</p>
<u>Native sequence</u>	<p>Amino acids Q518 – L875 (end residue) of human UBE3A. Residue Q230 of the fusion protein is equivalent to Q518 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<u>Protease cleavage</u>	TEV (<u>ENLYFQG</u>) residues 221 - 227
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pGEX 6P-1

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Nucleotide Sequence

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCA
CTCGACTTCTTTTGAATATCTTGAAGAAAAATATGAAGAGCATTTGTA
TGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGT
TTGGAGTTTCCCAATCTTCTTATTATATTGATGGTGATGTTAAATTAA
CACAGTCTATGGCCATCATACTGTTATATAGCTGACAAGCACAACATGTT
GGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCG
GTTTTGGATATTAGATACGGTGTTCGAGAATTGCATATAGTAAAGACT
TTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAA
AATGTTCGAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCAT
GTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTAT
ACATGGACCCAATGTGCTGGATGCGTTCCTCCAAAATTAGTTTTGTTTTAA
AAAACGTATTGAAGCTATCCACAAATTGATAAGTACTTGAAATCCAGC
AAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTG
GCGACCATCCTCCAAAATCGGATGAAAACCTGTATTTTCAGGGCgga t c
cCAGTTGAATCCATATTTGAGACTCAAAGTTAGACGTGACCATATCATA
GATGATGCACTTGTCCGGCTAGAGATGATCGCTATGGAAAATCCTGCAG
ACTTGAAGAAGCAGTTGTATGTGGAATTTGAAGGAGAACAAGGAGTTGA
TGAGGGAGGTGTTTCCAAAGAATTTTTTCAGCTGGTTGTGGAGGAAATC
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TTTGGTTTAATCCATCTTCTTTTGAAACTGAGGGTCAGTTTACTCTGAT
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CATTTTCCCATGGTTGTCTACAGGAAGCTAATGGGGAAAAAAGGAACTT
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GGCTATACCAGGGACTCTGTTCTGATTAGGGAGTTCTGGGAAATCGTTC
ATTCATTTACAGATGAACAGAAAAGACTCTTCTTGCAGTTTACAACGGG
CACAGACAGAGCACCTGTGGGAGGACTAGGAAAATTAAGATGATTATA
GCCAAAATGGCCCAGACACAGAAAGGTTACCTACATCTCATACTTGCT
TTAATGTGCTTTTACTTCCGGAATACTCAAGCAAAGAAAAACTTAAAGA
GAGATTGTTGAAGGCCATCACGTATGCCAAAGGATTTGGCATGCTGtaa