

# *MRCPPU REAGENTS and SERVICES*

## **Standard Operating Procedure**

### **Preparation of active UBE3A [1 – 875]**

**Enzyme description:-** UBE3A [1 - 875]

**Clone number:-** DU 25579

**Source:-** Recombinant

**Expression system:-** *E.coli*

**Tag:-** N-terminal GST

**Purification method:-** GSH Agarose

**Calculated molecular mass:-**

Monoisotopic 127, 284.83 daltons

Average Mass 127, 366.49 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 [1 - 875]

**Protein** UBE3A [1 - 875]

**Clone number** DU 25579

**Species** Human

**Accession number** Q05086-1

**Tags** N-terminal GST

**Bacterially  
expressed protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG  
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA  
VLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH  
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSS  
KYIAWPLQGWQATFGGGDHPPKSDENLYFQGGSM**MEK**LHQCYWKS**GEPQS**  
**DDIEASRMKRAAAKHLIERIYHQ**LTEGCGNEACTNEFCASCPTFLRMDN****  
**NAAAIKALELYKINAKLCDPHPSKKGASSAYLENSK**GAPNNSCSEIKMN****  
**KKGARIDFKDVTYL**TEEKVYEILELCREREDYSPLIRVIGRVFSSAEAL****  
**VQSF**RKVKQHTKEELKSLQAKDEDEKDEDEKEKAACSAAAMEEDSEASS  
RIGDSSQGDNNLQKLGPDVSVDAIRRVYTRLLSNEKIETAFNLALV  
YLSPNVECDLTYHNVYSRDPNYLNLFIIVMENRNLHSP**EYLEMALPLFC**  
**KAMSKLPLAAQGLIRLWSKYNADQIRRMET**FQQLITYKVISNEFN**SR**  
**NLVNDDDAIVAASKCLKMVYANVVGGEVDTNHNEEDDEEPIPESEL**T  
**LQELLG**EE**RNKKGPRVDPLETELGVKTLDCRKPLIP**FE**EFINEPLNEV**  
**LEM**DKDYTF**FK**VETENKFS**FM**TC**PF**ILNA**VT**KNLGLYDNRIRMY**SERR**  
**ITV**LYSLV**Q**QQLNPYLRLK**VRR**DHIID**DALVRLEM**IAMEN**PADL**KK**Q**L  
**YVE**FE**GE**QGVDEGGV**SKE**FFQ**L**VVEE**IF**NP**DIG**M**F**TYDE**ST**KL**F**W**F**N**PS**  
**S**F**ET**EG**Q**FT**L**IG**I**VL**GL**AI**Y**NNC**IL**D**V**H**F**PM**V**YR**K**LM**G**KK**G**T**FR**DL**GD**  
**S**HP**V**LY**Q**SL**K**DL**L**E**Y**EG**N**VED**DM**IT**F**Q**I**S**Q**T**D**L**F**GN**P**MM**Y**DL**K**ENG**D**K  
**I**P**I**T**N**EN**R**KE**F**V**N**LY**S**D**Y**IL**N**KS**V**E**K**Q**F**KA**F**RR**G**F**H**M**V**T**N**ES**P**L**K**Y**L**FR  
**P**EE**I**ELL**I**CG**S**R**N**LD**F**Q**A**LE**ET**TE**Y**D**G**GY**TR**DS**V**L**I**RE**F**WE**I**V**H**S**F**T**D**E  
**Q**K**R**L**F**L**Q**F**T**T**G**T**D**R**A**P**V**G**L**G**L**K**M**I**I**A**K**NG**P**D**T**ER**L**P**T**S**H**T**C**F**N**V**L**L**L**  
**P**E**Y**SS**K**E**K**L**K**ER**L**L**K**A**I**T**Y**A**K**G**F**G**M**L

**Native sequence** Amino acids M1 – L875 (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.

**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

ATGGAGAAGCTGCACCAGTGTATTATTGGAAATCAGGAGAACCTCAGTCTGACGACATTGA  
AGCTAGCCGAATGAAGCGAGCAGCTGCAAAGCATCTAATAGAACGCTACTACCACCAGT  
TAACTGAGGGCTGTGGAAATGAAGCCTGCACGAATGAGTTTTGTGCTTCCTGTCCAAC  
TTTCTTCGTATGGATAATAATGCAGCAGCTATTAAAGCCCTCGAGCTTTATAAGATTAA  
TGCAAAACTCTGTGATCCTCATCCCTCCAAGAAAGGAGCAAGCTCAGCTTACCTTGAGA  
ACTCGAAAGGTGCCCCCAACAACCTCCTGCTCTGAGATAAAAAATGAACAAGAAAGGCGCT  
AGAATTGATTTTTAAAGATGTGACTTACTTAACAGAAGAGAAGGTATATGAAATTCCTTGA  
ATTATGTAGAGAAAGAGAGGATTATCCCTTTAATCCGTGTTATTGGAAGAGTTTTTT  
CTAGTGCTGAGGCATTGGTACAGAGCTTCCGGAAAGTTAAACAACACACCAAGGAAGAA  
CTGAAATCTCTTCAAGCAAAAGATGAAGACAAAGATGAAGATGAAAAGGAAAAAGCTGC  
ATGTTCTGCTGCTGCTATGGAAGAAGACTCAGAAGCATCTTCCCTCAAGGATAGGTGATA  
GCTCACAGGGAGACAACAATTTGCAAAAATTAGGCCCTGATGATGTGTCTGTGGATATT  
GATGCCATTAGAAGGTCTACACCAGATTGCTCTCTAATGAAAAAATTGAACTGCCTT  
TCTCAATGCACTTGTATATTTGTCACCTAACGTGGAATGTGACTTGACGTATCACAATG  
TATACTCTCGAGATCCTAATTATCTGAATTTGTTCAATTATCGTAATGGAGAATAGAAAT  
CTCCACAGTCCTGAATATCTGGAAATGGCTTTGCCATTATTTTGCAAAGCGATGAGCAA  
GCTACCCCTTGACAGCCCAAGGAAAACCTGATCAGACTGTGGTCTAAATACAATGCAGACC  
AGATTCGGAGAATGATGGAGACATTTTCAGCAACTTATTACTTATAAAGTCATAAGCAAT  
GAATTTAACAGTCGAAATCTAGTGAATGATGATGATGCCATTGTTGCTGCTTCGAAGTG  
CTTGAAAATGGTTTACTATGCAAATGTAGTGGGAGGGGAAGTGGACACAAATCACAATG  
AAGAAGATGATGAAGAGCCCATCCCTGAGTCCAGCGAGCTGACACTTCAGGAACTTTTG  
GGAGAAGAAAGAAACAAGAAAGGTCTCGAGTGGACCCCTGGAAACTGAACTTGG  
TGTTAAAACCTGGATTGTGAAAACCACTTATCCCTTTTGAAGAGTTTATTAATGAAC  
CACTGAATGAGGTTCTAGAAATGGATAAAGATTATACTTTTTTCAAAGTAGAAACAGAG  
AACAAATTCCTTTTTATGACATGTCCCTTTATATTGAATGCTGTACAAAGAATTTGGG  
ATTATATTATGACAATAGAATTCGCATGTACAGTGAACGAAGAATCACTGTTCTCTACA  
GCTTAGTTCAAGGACAGCAGTTGAATCCATATTTGAGACTCAAAGTTAGACGTGACCAT  
ATCATAGATGATGCACTTGTCCGGCTAGAGATGATCGCTATGGAAAATCCTGCAGACTT  
GAAGAAGCAGTTGTATGTGGAATTTGAAGGAGAACAAGGAGTTGATGAGGGAGGTGTTT  
CCAAAGAATTTTTTCAGCTGGTTGTGGAGGAAATCTTCAATCCAGATATTGGTATGTTT  
ACATACGATGAATCTACAAAATTGTTTTGGTTAATCCATCTTCTTTTGAAACTGAGGG  
TCAGTTTACTCTGATTGGCATAGTACTGGGTCTGGCTATTTACAATAACTGTATACTGG  
ATGTACATTTTCCCATGGTTGTCTACAGGAAGCTAATGGGGAAAAAAGGAACTTTTCGT  
GACTTGGGAGACTCTCACCCAGTTCTATATCAGAGTTTTAAAGATTTATTGGAGTATGA  
AGGGAATGTGGAAGATGACATGATGATCACTTTCCAGATATCACAGACAGATCTTTTTG  
GTAACCCAATGATGTATGATCTAAAGGAAAATGGTGATAAAATTTCCAATTACAAATGAA  
AACAGGAAGGAATTTGTCAATCTTTATTCTGACTACATTCTCAATAAATCAGTAGAAAA  
ACAGTTCAAGGCTTTTCGGAGAGGTTTTTCATATGGTGACCAATGAATCTCCCTTAAAGT  
ACTTATTCAGACCAGAAGAAATTGAATTGCTTATATGTGGAAGCCGGAATCTAGATTTT  
CAAGCACTAGAAGAACTACAGAATATGACGGTGGCTATACCAGGGACTCTGTTCTGAT  
TAGGGAGTTCTGGGAAATCGTTCAATTTACAGATGAACAGAAAAGACTCTTCTTGC  
AGTTTACAACGGGCACAGACAGAGCACCTGTGGGAGGACTAGGAAAATTAAGATGATT  
ATAGCCAAAAATGGCCAGACACAGAAAGGTTACCTACATCTCATACTTGCTTTAATGT  
GCTTTTACTTCCGGAATACTCAAGCAAAAGAAAACTTAAAGAGAGATTGTTGAAGGCCA  
TCACGTATGCCAAAGGATTTGGCATGCTGtaa