

MRC PPU REAGENTS

Standard Operating Procedure

Preparation of UBLCP1 [1 – 318]

Enzyme description:- UBLCP1 [1 - 318]

Clone number:- DU 63523

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 63, 587.85 daltons

Average Mass 63, 628.81 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.90

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270mM sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

UBLCP1 [1 - 318]

<u>Protein</u>	UBLCP1 [1 - 318]
<u>Clone number</u>	DU 63523
<u>Species</u>	Human
<u>Accession number</u>	NM_145049.4
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPHYIDGDVKLTQSMALIRYIADKHNMLGGCPKERAELSMLEGA VLDIRYGVSRISYKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSMALPIIVKWGGQQEY SVTTLSEDDTVLDLKQFLKTLTGVLPERQKLLGLKVKGKPAENDVKLGA LKLKPNTKIMMMGTREESLEDVLGPPPDNDVVDFDIEDEVVEVENRE ENLLKISRVRKEYKVEILNPPREGKKLLVLDVDYTLFDHRSCAETGVEL MRPYLHEFLTSAYEDYDIVIWSATNMKWIEAKMKELGVSTNANYKITFM LDSAAMITVHTPRRGLIDVKPLGVIWGKFSEFYSKKNTIMFDDIGRNFL MNPQNGLKIRPFMKAHNLRDKDKELLKLTQYLKEIAKLDDFLDLNHKYW ERYLSKKQGQ
<u>Native sequence</u>	Amino acids M1 – Q318 (end) of human UBLCP1. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (LEVLFQGP) residues 221 – 228.
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I sites of pGex6P1

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Nucleotide Sequence Of Insert

ggatccATGGCTCTCCCTATCATTGTAAATGGGGTGGACAGGAGTATTCAGTGACCACAC
TTTCAGAAGATGATACTGTGCTCGATCTCAAACAGTTTCTCAAGACCCTTACAGGAGTTCT
TCCAGAACGCCAAAAGTTACTTGGACTCAAAGTTAAAGGCAAACCTGCAGAAAATGATGTT
AAGCTTGGAGCTCTCAAACCTGAAACCAAATACTAAAAATCATGATGATGGGAACTCGTGAGG
AGAGCTTGGAAAGATGTCTTAGGTCCACCCCCTGACAAATGATGATGTTGTTAATGACTTTGA
TATTGAAGATGAAGTAGTTGAAGTAGAAAATAGGGAAGAAAACCTACTGAAAATTTCTCGC
AGAGTGAAAGAGTACAAAGTGGAATTTTGAATCCTCCCAGGGAAGGGAAAAAGCTTTTGG
TGCTAGATGTTGATTATACATTATTTGACCACAGGTCTTGTGCAGAGACTGGGGTAGAATT
AATGCGGCCATATCTTCATGAATTTCTAACATCTGCCTATGAAGATTATGACATTGTTATT
TGGTCTGCAACAAATATGAAGTGGATTGAAGCTAAAAATGAAAGAGCTGGGAGTGAGCACAA
ATGCAAATTATAAGATTACTTTTCATGTTGGATAGTGCTGCTATGATAACAGTACATACTCC
AAGGAGAGGATTAATAGACGTAAAGCCTCTTGGTGTTATATGGGGAAAGTTTTCGGAGTTT
TACAGCAAGAAAAACACCATTATGTTTGATGACATAGGGAGAAATTTTCTAATGAACCCAC
AGAATGGACTAAAGATAAGGCCTTTTATGAAAGCGCACCTAAATCGTGATAAAGACAAAGA
ACTTTTAAAATTAATCAGTACCTCAAGGAGATAGCAAAATTAGATGACTTTTTTGGATCTA
AATCACAAATATTGGGAAAGATATCTCTCAAAGAAGCAAGGACAGtagcggccgc