

MRCPPU REAGENTS and SERVICES

Standard Operating Procedure

Preparation of USP30 [57 - 517]

Enzyme description:- USP30 [57 – 517]

Clone number:- DU 36294

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Agarose

Calculated molecular mass:-

Monoisotopic 79, 357.89 daltons

Average Mass 79, 409.00 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.83

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

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

Storage temperature:- -70 °C

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Clone Data Sheet

USP30 [57 - 517]

Protein USP30 [57 - 517]

Clone number DU 36294

Species Human

Accession number NM_032663.4

Tags N-terminal GST

**Bacterially
expressed protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAESMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFOGPLGSTERKKRRKG
LVPGLVNLGNTCFMNSLLQGLSACPAFIRWLEEFTSQYSRDQKEPPSH
QYLSLTLHLLKALSCQEVTDDEVLDASCLLDVLRMYRWQISSFEEQD
AHELFHVITSSLEDERDRQPRVTHLFDVHSLEQQSEITPKQITCRTRG
SPHPTSNHWKSQHPFHGRLTSNMVCKHCEHQSPVRFDTFDSL SLSIPA
ATWGHPLTLDHCLHFFISSESVRDVVDNCTKIEAKGTLNGEKVEHQ
TTFVKQLKLGKLPQCLCIHLQRLSWSSHGTPLKRHEHVQFNEFLMMDI
YKYHLLGHKPSQHNPKLNKNPGPTLELQDGPAPT PVLNQP GAPKTQI
FMNGACSPSLLPTLSAPMPFPLPVVPDYSSSTYLFRLMAVVVHHGDMH
SGHFVTYRRSPPSARNPLSTSNQWLWVSDDTVRKASLQEVLS S SAYLL
FYERVL SRMQHSQECKSEE

Native sequence Amino acids T57 – E517 (end) of human USP30.

Residue T232 of the fusion protein is equivalent to T57 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission site (LEVLFOGP) residues 221 – 228

Cloning sites *Bam*H1 and *Not*1 sites of pGex6P3

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**Nucleotide
sequence of
insert**

ggatccACAGAAAGAAAGAAGCGTAGAAAAGGGCTTGTGCCTGGCCTT
GTTAATTTAGGGAACACCTGCTTCATGAACTCCCTGCTACAAGGCCTG
TCTGCCTGTCCTGCTTTTCATCAGGTGGCTGGAAGAGTTCACCTCCCAG
TACTCCAGGGATCAGAAGGAGCCCCCTCACACCAGTATTTATCCTTA
ACACTCTTGCACCTTCTGAAAGCCTTGTCTGCCAAGAAGTTACTGAT
GATGAGGTCTTAGATGCAAGCTGCTTGTGGATGTCTTAAGAATGTAC
AGATGGCAGATCTCATCATTTGAAGAACAGGATGCTCACGAATTATTC
CATGTCATTACCTCGTCATTGGAAGATGAGCGAGACCGCCAGCCTCGG
GTCACACATTTGTTTGATGTGCATTCCCTGGAGCAGCAGTCAGAAATA
ACTCCCAAACAAATTACCTGCCGCACAAGAGGGTCACCTCACCCACA
TCCAATCACTGGAAGTCTCAACATCCTTTTCATGGAAGACTCACTAGT
AATATGGTCTGCAAACACTGTGAACACCAGAGTCCCTGTTTCGATTTGAT
ACCTTTGATAGCCTTTCACTAAGTATTCCAGCCGCCACATGGGGTCAC
CCATTGACCCTGGACCCTGCCTTACCCTTCATCTCATCAGAATCA
GTGCGGGATGTTGTGTGTGACAACGTACAAAGATTGAAGCCAAGGGA
ACGTTGAACGGGGAAAAGGTGGAACACCAGAGGACCCTTTTGTAA
CAGTTAAAACCTAGGGAAGCTCCCTCAGTGTCTCTGCATCCACCTACAG
CGGCTGAGCTGGTCCAGCCACGGCACGCCTCTGAAGCGGCATGAGCAC
GTGCAGTTCAATGAGTTCCTGATGATGGACATTTACAAGTACCACCTC
CTTGACATAAACCTAGTCAACACAACCCTAAACTGAACAAGAACCCA
GGGCCTACACTGGAGCTGCAGGATGGGCCGGGAGCCCCCACACCAGTT
CTGAATCAGCCAGGGGCCCCCAAACACAGATTTTTATGAATGGCGCC
TGCTCCCCATCTTTATTGCCAACGCTGTCAGCGCCGATGCCCTTCCCT
CTCCCAGTTGTTCCCGACTACAGCTCCTCCACATACCTCTTCCGGCTG
ATGGCAGTTGTCGTCCACCATGGAGACATGCACTCTGGACACTTTGTC
ACTTACCGACGGTCCCCACCTTCTGCCAGGAACCCTCTCTCAACTAGC
AATCAGTGGCTGTGGGTCTCCGATGACACTGTCCGCAAGGCCAGCCTG
CAGGAGGTCTGTCTCCAGCGCCTACCTGCTGTTCTACGAGCGCGTC
CTTCCAGGATGCAGCACCCAGAGCCAGGAGTGCAAGTCTGAAGAAtga
gcgcccgc