

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active USP46 [1 - 366] WDR48 [1 – 677]

<u>Enzyme description:-</u>	USP46 [1 - 366] WDR48 [1 – 677]
<u>Clone number:-</u>	DU 24347
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His6 - USP46, WDR48 - untagged
<u>Purification method:-</u>	Ni-NTA Agarose

Calculated molecular mass:-

Monoisotopic 44, 682.07 daltons (USP46) and 76, 210.47 (WDR48)
Average Mass 44, 710.77 daltons (USP46) and 76, 162.42 (WDR48)
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.47 (USP46) and 6.59 (WDR48)

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA,
10 mM DTT

Storage temperature:- -70 °C

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Clone Data Sheet

USP46 [1 - 366]

Protein USP46 [1 – 366]

Clone number DU 24347

Species Human

Accession number P62068 (USP46)

Tags N-terminal His6

**Baculovirus
expressed USP46
protein**

MGSSHHHHHSSGENLYFQGMTVRNIASICNMGTNASALEKDIG
PEQFPINEHYFGLVNFNGNTCYCNSVLQALYFCRPFRENVLAYKA
QQKKKENLLTCLADLFHSIATQKKKVGVI PPKKFISRLRKENDL
FDNYMQQDAHEFLNYLLNTIADILQEEKKQEKQNGKLKNGMNE
PAENNKPELTWVHEIFQGTLTNETRCLNCETVSSKDEDFLDLSV
DVEQNTSITHCLRDFSNTETLCSEQKYYCETCCSKQEAQKRMRV
KKLPMILALHLKRFKYMEQLHRYTKLSYRVVFPLELRLFNTSSD
AVNLDRMYDLVAVVVHCGSGPNRGHYITIVKSHGFWLLFDDDIV
EKIDAQAIEEFYGLTSDISKNSESGYILFYQSRE

Native sequence Amino acids M1 – E366 (end residue) of human USP46.
Residue M21 of the fusion protein is equivalent to M1 of the native
enzyme. The His6 tag is located at residues 5 - 10.

Protease cleavage rTEV (ENLYFQ) residues 14 - 19

Cloning sites *Bam*HI and *Not*I sites of pFastBac Dual.

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Nucleotide Sequence USP46

ggatccATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCGAAAA
CCTGTATTTTCAGGGCATGACTGTCCGAAACATCGCCTCCATCTGTAATA
TGGGCACCAATGCCTCTGCTCTGGAAAAAGACATTGGTCCAGAGCAGTTT
CCAATCAATGAACACTATTTTCGGATTGGTCAATTTTGGAAACACATGCTA
CTGTAACTCCGTGCTTCAGGCATTGTACTTCTGCCGTCCATTCCGGGAGA
ATGTGTTGGCATAACAAGGCCAGCAAAAAGAAGAAGGAAAACCTTGCTGACG
TGCCTGGCGGACCTTTTCCACAGCATTGCCACACAGAAGAAGAAGGTTGG
CGTCATCCCACCAAGAAGTTCATTTCAAGGCTGAGAAAAGAGAATGATC
TCTTTGATAACTACATGCAGCAGGATGCTCATGAATTTTTTAAATTATTG
CTAAACACTATTGCGGACATCCTTCAGGAGGAGAAGAAACAGGAAAAACA
AAATGGAAAATTAATAAATGGCAACATGAACGAACCTGCGGAAAATAATA
AACCAGAACTCACCTGGGTCCATGAGATTTTTTCAGGGAACGCTTACCAAT
GAAACTCGATGCTTGAACGTGAAACTGTTAGTAGCAAAGATGAAGATTT
TCTTGACCTTTCTGTTGATGTGGAGCAGAATACATCCATTACCCACTGTC
TAAGAGACTTCAGCAACACAGAAACACTGTGTAGTGAACAAAAATATTAT
TGTGAAACATGCTGCAGCAAACAAGAAGCCAGAAAAGGATGAGGGTAAA
AAAGCTGCCCATGATCTTGGCCCTGCACCTAAAGCGGTTCAAGTACATGG
AGCAGCTGCACAGATAACCAAGCTGTCTTACCGTGTGGTCTTCCCTCTG
GAACTCCGGCTCTTCAACACCTCCAGTGATGCAGTGAACCTGGACCGCAT
GTATGACTTGGTTGCGGTGGTCTTCACTGTGGCAGTGGTCCTAATCGTG
GGCATTATATCACTATTGTGAAAAGTCACGGCTTCTGGCTTTTGTTTGAT
GATGACATTGTAGAGAAAATAGATGCTCAAGCTATTGAAGAATTCATATGG
CCTGACGTCAGATATATCAAAAAATTCAGAATCTGGATATATTTTATTCT
ATCAGTCAAGAGAGtaagcggccgc

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WDR48 [1 – 677]

Protein WDR48 [1 – 677]

Clone number DU 24373

Species Human

Accession number Q8TAF3 (WDR48)

Tags Untagged WDR48

**Baculovirus
expressed WDR48
protein**

MAAHRQNTAGRRKVQVS YVIRDEVEKYNRNGVNALQLDPALNR
LFTAGRDSIIRISVSNQHKQDPYIASMEHHTDWVNDIVLCCNGK
TLISASSD TTVKVNNAHKGFCMSTLRTHKD YVKALAYAKDKELV
ASAGLDRQIFLWDVNTLTALTASNNTVTTSSLSGNKDSIYSLAM
NQLGTII VSGSTEKVLRVWDPRTC AKLMKLGHTDNVKALLNR
DGTQCLSGSSDGTIRLWLSLGGQRCIATYRVHDEGVWALQVND AF
THVYSGGRDRKIYCTDLRNPDIRVLI CEEKAPVLKMELDRSADP
PPAIWVATTKSTVNKWT LKGIHNFRA S GDYDNDCTNPITPLCTQ
PDQVIKGGASIIQCHILNDRHILTKDTNNNVAYWDVLKACKVE
DLGKVD FEDEIKKRFKMVYV PNWFSVDLKTGMLTITLDES DCFA
AWVSAKDAGFSSPDGSDPKLNLGGLLLQALLEYWPRTHVNP MDE
EENEVNHVNGEQENRVQKNGYFQVPPHTPVIFGEAGGRTLFRL
LCRDSGGETESMLLNETVPQWVIDITVDKNMPKFNKIPFYLQPH
ASSGAKTLKKDRLSASDMLQVRKVM EHVYEKIIINLDNESQTTSS
SNNEKPGEQEKEEDIAVLAEEKIELLCQDQVLDPNMDLRTVKHF
IWKSGGDLTLHYRQKST

Native sequence Amino acids M1 – T677 (end residue) of human WDR48.

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Nucleotide Sequence WDR48

gctagcATGGCGGCCCATCACCGGCAGAACACAGCAGGGCGGAGGAAAGTGCAGGTTTCC
TATGTTATTCGAGATGAAGTGGAGAAGTACAACCGAAATGGAGTCAATGCTCTGCAGCTG
GATCCAGCACTAAATAGACTTTTCACAGCCGGTCGAGACTCTATCATAAGAATATGGAGT
GTCAATCAGCACAAGCAAGATCCATATATAGCATCTATGGAACACCATACTGATTGGGTA
AACGACATTGTACTCTGTTGTAATGGGAAAACATTAATATCTGCTTCTTCTGACACGACA
GTAAGATGGAATGCACACAAGGGATTTTGCATGTCAACATTAAGGACACATAAGGAT
TACGTAAAGGCCTTAGCATATGCCAAGGATAAAGAAGTAGTAGCATCAGCTGGGTTGGAC
AGACAAATATTCCTTTGGGATGTGAATACTCTAACAGCATTGACTGCCCTCAAATAACACT
GTCACAACCTTCTTCTTTAAGTGGAAACAAGATTCCATTTATAGCCTGGCCATGAATCAA
CTGGGAACAATCATTGTATCAGGGTCCACTGAAAAGGTGTTACGGGTATGGGATCCAAGA
ACATGTGCAAACTAATGAAGCTTAAAGGGCACACGGATAATGTGAAGGCATTGCTATTA
AACAGAGATGGCAGCAATGCCTGTCAGGCAGTTCCTGATGGGACAATTCGCCTTTGGTCC
CTTGGCCAGCAGAGATGTATAGCAACATACCGAGTCCATGATGAAGGTGTTTGGGCGCTG
CAAGTCAATGATGCCTTCACACATGTGTATTCTGGTGGAAAGGGACAGGAAGATTTATTGT
ACAGACCTAAGAAACCCTGACATTCGGGTGCTAATTTGTGAAGAAAAAGCACCAGTTCTC
AAGATGGAGCTTGATAGATCAGCTGATCCTCCTCCTGCAATTTGGGTGCAACAACCTAAG
TCTACAGTAAATAAATGGACTTTGAAAGGAATTCATAATTTTAGAGCCTCTGGAGATTAT
GACAATGACTGTACAAATCCTATAACACCTCTTTGTACACAACCTGACCAGGTTATTA
GGGGGTGCTAGTATTATTAGTGCCACATTCTTAATGATAAGAGACATATATTAACCAA
GATACCAATAAATAATGTGGCATATTGGGATGTATTGAAGGCATGTAAAGTTGAAGATCTG
GGCAAAGTGGATTTTGAAGATGAAATTAAGAAAAGATTTAAAATGGTGTATGTGCCAAAT
TGGTTCTCAGTAGACTTAAAAACAGGGATGTTAACTATTACTTTGGATGAAAGTGATTGT
TTTGCTGCCTGGGTTTCTGCAAAAGATGCTGGTTTTAGCAGCCCTGATGGGTGAGATCCA
AAATTGAATTTAGGAGGACTTTTACTCCAAGCACTCCTGGAATATTGGCCTAGAACACAT
GTGAATCCAATGGATGAAGAGGAAAATGAAGTAAACCATGTAAATGGGGAGCAGGAGAAC
CGAGTGCAGAAGGGAAAATGGATATTTTCAAGTGCCCCCACATACACCCGTGATCTTTGGT
GAAGCTGGAGGTGCGCACACTGTTCAAGCTGCTCTGCCGAGATTCCGGGGGTGAGACTGAG
TCTATGCTTCTTAATGAAACAGTGCCACAATGGGTAATTGACATCACTGTGGATAAAAAAT
ATGCCCAAATTCACAAAATTCCTTTCTACCTCCAACCTCATGCATCTTCAGGAGCAAAA
ACCTTAAAAAAGATAGACTCTCTGCTAGTGACATGCTCCAAGTCCGAAAAGTTATGGAA
CATGTTTATGAAAAAATTATCAACTTGGATAATGAGTCTCAAACCACTAGCTCTTCTAAT
AATGAAAAACCAGGAGAACAGGAAAAAGAAGAAGATATTGCTGTGTTGGCAGAGGAGAAA
ATTGAACCTTTTGTGCCAAGACCAGGTTTTGGATCCAAATATGGACCTTCGAACAGTGAAA
CACTTCATATGGAAGAGCGGTGGAGACCTCACCTCCATTACCGTCAGAAGTCCACGtga
gtacc