

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active USP12 [1 - 370] WDR48 [1 – 677]

<u>Enzyme description:-</u>	USP12 [1 - 370] WDR48 [1 – 677]
<u>Clone number:-</u>	DU 24373
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His6 - USP12, WDR48 - untagged
<u>Purification method:-</u>	Ni-NTA Agarose

Calculated molecular mass:-

Monoisotopic 45, 097.24 daltons (USP12) and 76, 210.47 (WDR48)
Average Mass 45, 126.16 daltons (USP12) and 76, 162.42 (WDR48)
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.28 (USP12) 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

USP12 [1 - 370]

Protein USP12 [1 – 370]

Clone number DU 24373

Species Human

Accession number O75317 (USP12)

Tags N-terminal His6

**Baculovirus
expressed USP12
protein**

MGSSHHHHHHSSGENLYFQGM EILMTVSKFASICTMGANASALE
KEIGPEQFPVNEHYFGLVNF GNTCYCNSVLQALYFCRPFREKVL
AYKSQPRKESLLTCLADLFHSIATQKKKVGVI PPKKFITRLRK
ENELFDNYMQQDAHEFLNYLLNTIADILQEERKQEKQNGRLPNG
NIDNENNNSTPDPTWVHEIFQGTLTNETRCLTCETISSKDEDFL
DLSVDVEQNTSITHCLRGFSNTETLCSEYKYYCEE CRSKQEAHK
RMKVKKLPMILALHLKRFKYMDQLHRYTKLSYRVVFPLELRLFN
TSGDATNPDRMYDLVAVVVHCGSGPNRGHYIAIVKSHDFWLLFD
DDIVEKIDAQAIEEFYGLTSDISKNSESGYILFYQSR

Native sequence Amino acids M1 – R370 (end residue) of human USP12.
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 BamHI and NotI sites of pFastBac Dual.

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Nucleotide Sequence USP12

ggatccATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCGAAAACCTGTATTTT
CAGGGCATGGAAATCCTAATGACAGTCTCCAAATTCGCCTCCATCTGTACCATGGGCGCC
AATGCTTCGGCATTAGAGAAAGAGATTGGTCCAGAACAGTTTCCGGTCAATGAGCACTAT
TTTGGATTAGTCAATTTTGGGAATACCTGCTACTGCAATTCAGTTCTTCAAGCACTTTAT
TTTTGTCTCCATTTTCGGGAAAAAGTTCTTGCCTATAAGAGTCAACCTAGGAAAAAGGAG
AGCCTTCTTACATGCTTAGCAGATCTCTTCCATAGCATAGCCACTCAGAAGAAAAAGGTT
GGAGTAATACCCCCTAAGAAGTTCATCACAAGATTACGGAAAGAAAAATGAGCTTTTTGAC
AACTACATGCAACAAGATGCCCATGAATTCCTTAAATTACCTACTAAATACAATTGCTGAT
ATTTTACAAGAAGAGAGAAAGCAGGAAAAACAAAATGGTCGTTTACCTAATGGTAATATT
GATAATGAAAATAATAACAGCACACCAGACCCAACGTGGGTTTCATGAGATTTTTTCAGGGA
ACATTAACATAATGAAACCAGATGTCTTACTTGTGAAACTATAAGCAGCAAAGATGAAGAT
TTTTTAGACCTTTCTGTTGACGTGGAACAAAATACATCAATTACTCACTGCTTAAGGGGT
TTCAGCAACACAGAACTCTGTGCAGTGAATACAAGTATTACTGTGAAGAGTGTCGCAGC
AAACAGGAAGCACACAAACGGATGAAAGTTAAAAAACTGCCCATGATTCTAGCTCTACAC
CTGAAGAGATTTAAATATATGGATCAACTTCATCGATATACAAAACCTCTTACC GGTA
GTTTTTCCTTTAGAACTTCGTCTGTTTAACTTCAGGTGATGCCACCAATCCAGACAGA
ATGTACGACCTTGTGCTGTTGTGGTTCACTGTGGAAGTGGTCCAATCGAGGCCATTAT
ATTGCAATAGTTAAGAGTCATGATTTTTGGTTGTTGTTGATGACGACATTGTAGAAAAA
ATAGATGCACAAGCTATTGAAGAATTCTACGGGTTGACATCAGATATCTCAAAGAACTCT
GAGTCTGGTTACATCCTTTTCTATCAGTCTCGGGACTgagcggccgc

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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**

MAAHRQNTAGRRKQVSYVIRDEVEKYNRNGVNALQLDPALNR
LFTAGRDSIIRIWSVNQHKQDPYIASMEHHTDWVNDIVLCCNGK
TLISASSDTTVKVNNAHKGFCMSTLRTHKDYVKALAYAKDELV
ASAGLDRQIFLWDVNTLTALTASNNTVTTSSLSGNKDSIYSLAM
NQLGTIIVSGSTEKVLRVWDPRTC AKLMKLGHTDNVKALLNR
DGTQCLSGSSDGTIRLWSLQQRCIATYRVHDEGVWALQVND AF
THVYSGGRDRKIYCTDLRNPDIRVLI CEEKAPVLKMELDRSADP
PPAIWVATTKSTVNKWT LKGIHNF RASGDYDNDCTNPITPLCTQ
PDQVIKGGASIIQCHILNDRHILTKDTNNNVAYWDVLKACKVE
DLGKVDFEDEIKKRFKMVYVPNWF SVDLKTGMLTITLDESDF A
AWVSAKDAGFSSPDGSDPKLNLGGLLQALLEYWP RTHVNPMD E
EENEVNHVNGEQENRVQKNGYFQVPPHTPVI FGEAGGRTLFRL
LCRDSGGETESMLLNETVPQWVIDITVDKNMPKFNKIPFYLOPH
ASSGAKTLKDRLSASDMLQVRKVMHVYEKI INLDNESQTTSS
SNNEKPGEQEKEEDIAVLAEKIELLCQDQVLDPNMDLRTVKHF
IWKSGGDLTLHYRQKST

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

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Nucleotide Sequence WDR48

gctagcATGGCGGCCCATCACCGGCAGAACACAGCAGGGCGGAGGAAAGTGCAGGTTTCC
TATGTTATTCGAGATGAAGTGGAGAAGTACAACCGAAATGGAGTCAATGCTCTGCAGCTG
GATCCAGCACTAAATAGACTTTTCACAGCCGGTTCGAGACTCTATCATAAGAATATGGAGT
GTCAATCAGCACAAGCAAGATCCATATATAGCATCTATGGAACACCATACTGATTGGGTA
AACGACATTGTACTCTGTTGTAATGGGAAAACATTAATATCTGCTTCTTCTGACACGACA
GTAAGATATGGAATGCACACAAGGGATTTTGCATGTCAACATTAAGGACACATAAGGAT
TACGTAAAGGCCTTAGCATATGCCAAGGATAAAGAAGCTAGTAGCATCAGCTGGGTTGGAC
AGACAAATATTCCTTTGGGATGTGAATACTCTAACAGCATTGACTGCCCTCAAATAACACT
GTCACAACCTTCTTCTTTAAGTGGAAACAAGATTCCATTTATAGCCTGGCCATGAATCAA
CTGGGAACAATCATTGTATCAGGGTCCACTGAAAAGGTGTTACGGGTATGGGATCCAAGA
ACATGTGCAAACTAATGAAGCTTAAAGGGCACACGGATAATGTGAAGGCATTGCTATTA
AACAGAGATGGCAGCAATGCCTGTCAGGCAGTTCCTGATGGGACAATTCGCCTTTGGTCC
CTTGGCCAGCAGAGATGTATAGCAACATACCGAGTCCATGATGAAGGTGTTTGGGCGCTG
CAAGTCAATGATGCCTTCACACATGTGTATTCTGGTGGAAAGGGACAGGAAGATTTATTGT
ACAGACCTAAGAAACCCTGACATTCGGGTGCTAATTTGTGAAGAAAAAGCACCAGTTCTC
AAGATGGAGCTTGATAGATCAGCTGATCCTCCTCCTGCAATTTGGGTGCAACAACCTAAG
TCTACAGTAAATAAATGGACTTTGAAAGGAATTCATAATTTTAGAGCCTCTGGAGATTAT
GACAATGACTGTACAAATCCTATAACACCTCTTTGTACACAACCTGACCAGGTTATTA
GGGGGTGCTAGTATTATTAGTGCCACATTCTTAATGATAAGAGACATATATTAACCAA
GATACCAATAAATAATGTGGCATATTGGGATGTATTGAAGGCATGTAAAGTTGAAGATCTG
GGCAAAGTGGATTTTGAAGATGAAATTAAGAAAAGATTTAAAATGGTGTATGTGCCAAAT
TGGTTCTCAGTAGACTTAAAAACAGGGATGTTAACTATTACTTTGGATGAAAGTGATTGT
TTTGCTGCCTGGGTTTCTGCAAAAGATGCTGGTTTTCAGCAGCCCTGATGGGTGAGATCCA
AAATTGAATTTAGGAGGACTTTTACTCCAAGCACTCCTGGAATATTGGCCTAGAACACAT
GTGAATCCAATGGATGAAGAGGAAAATGAAGTAAACCATGTAAATGGGGAGCAGGAGAAC
CGAGTGCAGAAGGGAAAATGGATATTTTCAAGTGCCCCCACATACACCCGTGATCTTTGGT
GAAGCTGGAGGTGCGCACACTGTTCAAGCTGCTCTGCCGAGATTCCGGGGGTGAGACTGAG
TCTATGCTTCTTAATGAAACAGTGCCACAATGGGTAATTGACATCACTGTGGATAAAAAAT
ATGCCCAAATTCACAAAATTCCTTTCTACCTCCAACCTCATGCATCTTCAGGAGCAAAA
ACCTTAAAAAAGATAGACTCTCTGCTAGTGACATGCTCCAAGTCCGAAAAGTTATGGAA
CATGTTTATGAAAAAATTATCAACTTGGATAATGAGTCTCAAACCACTAGCTCTTCTAAT
AATGAAAAACCAGGAGAACAGGAAAAAGAAGAAGATATTGCTGTGTTGGCAGAGGAGAAA
ATTGAACTTTTGTGCCAAGACCAGTTTTGGATCCAAATATGGACCTTCGAACAGTGAAA
CACTTCATATGGAAGAGCGGTGGAGACCTCACCTCCATTACCGTCAGAAGTCCACGtg
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