

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-USP1 / UAF1

Enzyme description:- His-USP1 (1-785) [G670A/G671A]
UAF1 (1-732)

Clone number:- DU23056

Source:- Recombinant

Tag:- N-terminal His

Purification method:- Ni-NTA-agarose

Expression level:- 3mg/L

Calculated molecular mass:- USP1

Monoisotopic 90447
Average Mass 90502
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.67

Calculated molecular mass:- UAF1

Monoisotopic 82547
Average Mass 82597
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.87

Purity:- >80%

Enzyme storage buffer:-

50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT

Storage temperature:- -80°C

Assay:-

Active against Ub-Rho-Gly. Very sensitive to freeze thawing

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Clone Data Sheet

Protein name USP1 (1-785) [G670A, G671A]

<u>Protein</u>	USP1 (1-785) FL [G670A, G671A] prevents autocleavage
<u>Synonyms</u>	UBP1
<u>Clone Number</u>	DU23056
<u>Species</u>	Human
<u>Accession Number</u>	O94782
<u>Tags</u>	N-terminal His6-
Aminoacid sequence of the expressed protein	MGSSHHHHHSSGENLYFQGMPGVIPSESNGLSRGSPSKK NRLSLKFFQKKETKRALDFTDSQENEEKASEYRASEIDQV VPAAQSSPINCEKRENLLPFVGLNNLGNTCYLNSILQVLY FCPGFKSGVKHLFNIISRKKEALKDEANQKDKGNCKEDSL ASYELICSLQSLIISVEQLQASFLLNPEKYTDELATQPRR LLNTLRELNPMYEGYLQHDAQEVLQCILGNIQETCQLLKK EEVKNVAELPTKVEEIPHPKEEMNGINSIEMDSMRHSEDF KEKLPKGNKGRKSDTEFGNMKKKVKLSKEHQSLLENQRQT RSKRKATSDTLESPPKIIPKYISENESPRPSQKKSrvKIN WLKSATKQPSILSKFCSLGKITTNQGVKGQSKENECdPEE DLGKCESDNTTNGCGLESPGNTVTPVNVNEVKPINKGEEQ IGFELVEKLFQQLVLRTRCLECESLTERREDFQDISVPV QEDELSKVEESSEISPEPKTEMKTLRWAI SQFASVERIVG EDKYFCENCHHYTEAERSLLFDKMPEVITIHLCFAASGL EFDCYGGGLSKINTPLLTPLKLSLEEWSTKPTNDSYGLFA VVMHSGITISSGHYTASVKVTDLNSLELDKGNFVVDQMC IGKPEPLNEEEARGVVENYNDEEVSIRVGGNTQPSKVLNK KNVEAIGLLAAQKSKADYELYNKASNPDKVASTAFAENRN SETSDTTGTHESDRNKESDQTGINISGFENKISYVVQSL KEYEGKLLFDSEVKVTEEKDFLNSLSPSTSPTSTPYLL FYKKL
Native sequence	in bold
Protease cleavage	TEV protease site underlined
Cloning sites	BamH1 / NotI

DNA sequence of insert

GGATCCATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCG
AAAACCTGTATTTTCAGGGCATGCCTGGTGTACATACCTAGTGAAAG
TAATGGACTTTCAAGAGGTAGCCCTTCAAAGAAAAACAGACTTTCC
TTAAAGTTTTTTCAGAAAAAGGAACTAAGAGAGCTTTGGATTTCA
CAGATTCTCAAGAAAATGAAGAAAAGCTTCTGAATATAGAGCATC
TGAAATTGATCAAGTTGTTCCCTGCAGCACAGTCTTCACCTATAAAC
TGTGAGAAGAGAGAAAACTTGTACCATTTGTGGGACTGAATAATC
TCGGCAATACTTGTATCTTAATAGTATACTTCAGGTATTATATTT
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TCAAGGAAGAAAGAAGCTCTAAAGGATGAAGCCAATCAAAAAGACA
AGGGAAATTGCAAAGAAGATTCTTTGGCAAGTTATGAATTGATATG
CAGTTTACAGTCCCTAATCATTTCCGGTTGAACAGCTCCAGGCTAGT
TTTCTCTTAAATCCAGAGAAATATACTGATGAACTTGCCACTCAGC
CAAGGCGACTGCTTAACACACTGAGGGAACCAACCCTATGTATGA
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GAAAGAGGAAATGAATGGTATTAACAGCATAGAGATGGACAGTATG
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CCTCTAATCCTGATAAGGTTGCTAGTACAGCGTTTGTGAAAATAG
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GAAGTGGTTGCTTTTTGATGATTCTGAAGTCAAAGTTACTGAAGAG
AAGGACTTTCTGAATTCTCTTTCCCTTCTACATCTCCTACTTCTA
CTCCTTACTTGCTATTTTATAAGAAATTATAGGCGGCCGC

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Clone Data Sheet

Protein name UAF1 (1-732)

Protein UAF1
Synonyms WDR48
Clone Number DU23056
Species Human
Accession Number Q8TAF3
Tags n/a

Aminoacid sequence of the
expressed protein

**MAAHRQNTAGRRKVQVS YVIRDEVEKYNRNGVNALQLDP
ALNRLF TAGRDSI IRIWSVNQHKQDPY IASMEHHTDWVND
IVLCCNGKTLISASSDTTVKVWNAHKGFCMSTLRTHKDYV
KALAYAKDELVASAGLDRQIFLWDVNTLTALTASNNTVT
TSSLSGNKDSIYSLAMNQLGTII VSGSTEKVLRVWDPRTC
AKLMKLGHTDNVKALLLN RDGTQCLSGSSDGTIRLWSLG
QQRCIATYRVHDEGVWALQVNDAFTHVYSGGRDRKIYCTD
LRNPDIRVLI CEEKAPVLKME LDRSADPPPAIWVATTKST
VNKWT LKGIHNF RASGDYDNDCTNPITPLCTQPDQVIKGG
ASIIQCHILNDRHILTKDTNNNVAYWDVLKACKVEDLGK
VDFEDEIKKRFKMVYVPNWF SVDLKTGMLTITLDESDF
AWVSAKDAGFSSPDGSDPKLNLGGLLQALLEYWPRTNV
PMDEEENEVNHVNGEQENRVQKNGYFQVPPHTPVIFGEA
GGRTLFRLLCRDSGGETESMLLNETVPQWVIDITVDKNMP
KFNKIPFYLQPHASSGAKTLKDRLSASDMLQVRKVMHV
YEKIINLDNESQTTSSSNNEKPGEQEKEEDIAVLAEKIE
LLCQDQVLDPNMDLRTVKHF IWKSGGDLTLHYRQKST**

Native sequence in bold

DNA sequence of cassette

ATGGCGGCCCATCACCGGCAGAACACAGCAGGGCGGAGGAAAGTGC
AGGTTTCCTATGTTATTCGAGATGAAGTGGAGAAGTACAACCGAAA
TGGAGTCAATGCTCTGCAGCTGGATCCAGCACTAAATAGACTTTTC
ACAGCCGGTCGAGACTCTATCATAAGAATATGGAGTGTCAATCAGC
ACAAGCAAGATCCATATATAGCATCTATGGAACACCATACTGATTG
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