USP1 [6His-tagged] / **UAF1** [untagged]

Deconjugating enzyme: Deubiquitylase

Alternate Names: USP1 = Deubiquitinating enzyme 1, hUBP, Ubiquitin carboxyl terminal hydrolase 1, Ubiquitin specific processing protease 1, Ubiquitin thiolesterase 1, UBP; UAF1 = WD repeat endosomal protein, KIAA1449, P80, p80 endosomal protein

Cat. No. **64-0040-050** Quantity: 50 µg
Lot. No. **30139** Storage: -70˚C

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CERTIFICATE OF ANALYSIS Page 1 of 2

Background

Deconjugating enzymes (DCEs) are proteases that process ubiquitin or ubiquitin-like gene products, reverse the modification of proteins by a single ubiquitin or ubiquitin-like protein (UBL) and remodel polyubiquitin (or poly-UBL) chains on target proteins (Reyes-Turcu *et al.,* 2009). The deubiquitylating – or deubiquitinating – enzymes (DUBs) represent the largest family of DCEs and regulate ubiquitin-dependent signalling pathways. The activities of the DUBs include the generation of free ubiquitin from precursor molecules, the recycling of ubiquitin following substrate degradation to maintain cellular ubiquitin homeostasis and the removal of ubiquitin or ubiquitin-like proteins (UBL) modifications through chain editing to rescue proteins from proteasomal degradation or to influence cell signalling events (Komander *et al.,* 2009). There are two main classes of DUB, cysteine proteases and metalloproteases. Ubiquitin specific protease 1 (USP1) is a member of the cysteine protease enzyme family and cloning of the human gene was first described by Fujiwara *et al.* (1998). USP1 has been shown to act as a negative regulator of the Fanconi anaemia pathway. Inhibition of USP1 by siRNA knockdown increased the monoubiquitylation of the Fanconi anaemia effector protein, FANCD2, and increased cellular resistance to DNA cross-linking agents, suggesting that USP1 deubiquitylates FANCD2 (Huang *et al.,* 2006). USP1 associated factor 1 (UAF1) not only interacts with and stabilizes USP1 but also stimulates its enzymatic activity. UAF1 binding increases the catalytic

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Physical Characteristics

Species: human

Source: Insect Sf21

Quantity: 50 µg

Concentration: 0.5 mg/ml

Formulation: 50 mM HEPES pH 7.5,
150 mM sodium chloride,
2 mM dithiothreitol, 10% glycerol

Molecular Weight: USP1 = 90.5 kDa
UAF1 = 76.2 kDa

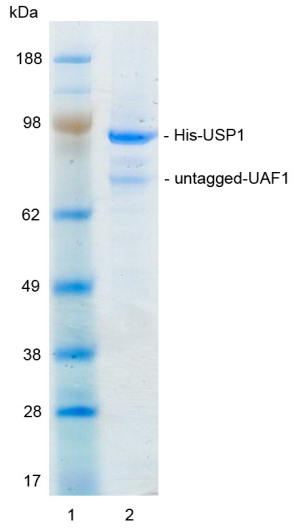
Purity: >90% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70˚C; Ubiquigent strongly recommends aliquoting for single use as required

Protein Sequences: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg His-USP1/untagged-UAF1



Protein Identification:
Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay:
The activity of His-USP1/untagged-UAF1 was validated by determining the increase in fluorescence measured as a result of the enzyme catalysed cleavage of the fluorogenic substrate Ubiquitin-Rhodamine110-Glycine generating Ubiquitin and Rhodamine110-Glycine. Incubation of the substrate in the presence or absence of His-USP1/untagged-UAF1 was compared confirming the deubiquitylating activity of His-USP1/untagged-UAF1.

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Lot-specific COA version tracker: v1.0.0

USP1 [6His-tagged] / UAF1 [untagged]

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Cat. No.	**64-0040-050**	Quantity:	50 µg
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Background

Continued from page 1

turnover (Kcat) but does not increase the affinity of the USP1 enzyme for the substrate (Km) (Cohn *et al.,* 2007). To explore USP1 autocleavage, a USP1 variant was expressed where the amino acids at positions 670 and 671 were changed from Gly-Gly to Ala-Ala. This mutation inhibited both the cleavage and degradation of USP1 (Huang *et al.,* 2006). USP1 plays important roles in cancer-related processes, such as the DNA damage response, and the maintenance of the undifferentiated state of osteosarcoma cells. Inhibiting the function of the USP1/UAF1 complex sensitizes cancer cells to chemotherapy, suggesting that this complex is a relevant anticancer target (Garcia-Santisteban *et al.,* 2012).

References:

Cohn MA, Kowal P, Yang K, Haas W, Huang TT, *et al.* (2007) A UAF1-containing multisubunit protein complex regulates the Fanconi anemia pathway. *Mol Cell* **28**, 786-797.

Fujiwara T, Saito A, Suzuki M, Shinomiya H, Suzuki T, Takahashi E, *et al.* (1998) Identification and chromosomal assignment of USP1, a novel gene encoding a human ubiquitin-specific protease. *Genomics* **54**, 155-158.

Garcia-Santisteban I, Zorroza K and Rodriguez JA (2012) Two nuclear localization signals in USP1 mediate nuclear import of the USP1/UAF1 complex. *PloS one* **7**, e38570.

Huang TT, Nijman SM, Mirchandani KD, Galardy PJ, Cohn MA, Haas W, *et al.* (2006) Regulation of monoubiquitinated PCNA by DUB autocleavage. *Nature Cell Biology* **8**, 339-347.

Komander D, Clague MJ and Urbe S (2009) Breaking the chains: structure and function of the deubiquitinases. *Nat Rev Mol Cell Biol* **10**, 550-563.

Reyes-Turcu FE, Ventii KH and Wilkinson KD (2009) Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. *Ann Review Biochem* **78**, 363-397.

Physical Characteristics

Continued from page 1

USP1 Protein Sequence:

M G S S H H H H H H S S G E N L Y F Q G
MPGVIPSESNGLSRGSPSKKNRLSLKFFQK
KETKRALDFTDSQENEEKASEYRA
SEIDQVVPAAQSSPINCEKRENLLPFVGLNN
LGNTCYLNSILQVLYFCPGFKSGVKHLFNI
ISRKKEALKDEANQKDKGNCKEDSLASYEL
ICSLQSLIISVEQLQASFLLNPEKY
TDELATQPRRLLNTLRELNPMYEGYLQH
DAQEVLQCILGNIQETCQLLKKEEVKN
VAELPTKVEEIPHPKEEMNGINSIEMDSMRH
SEDFKEKLPKGNGKRKSDTEFGNMKKKVKL
SKEHQSLEENQRQTRSKRKATSDTLESPPKI
IPKYISENESPRPSQKKSRVKINWLKSAT
KQPSILSKFCSLGKITTNQGVKGQSKENECD
PEEDLGKCESDNTTNGCGLESPGNTVTPVN
VNEVKPINKGEEQIGFELVEKLFQGQLVLR
TRCLECESLTERREDFQDISVPVQEDELSK
VEESSEISPEPKTEMKTLRWAISQFASVER
IVGEDKYFCENCHHYTEAERSLLFDKMPE
VITIHLKCFAASGLEFDCYGGGLSKINT
PLLTPLKLSLEEWSTKPTNDSYGLFAV
VMHSGITISSGHYTASVKVTDLNSLELD
KGNFVVDQMCEIGKPEPLNEEEARGVVE
NYNDEEVSIRVGGNTQPSKVLNKKNVEAIGL
LAAQKSKADYELYNKANPDKVASTAFAENRN
SETSDTTGTHESDRNKESSDQTGINISGFEN
KISYVVQSLKEYEGKWLLFDDSEVKVTEEKD
FLNSLSPSTSPTSTPYLLFYKKL

Tag **(bold text)**: N-terminal His
Protease cleavage site: TEV™ (ENLYFQ▼G)
USP1 (regular text): Start ***bold italics*** (amino acid residues 1-785)
Accession number: NP_003359*

* Except for two mutations (G670A, G671A) introduced to prevent USP1 autocleavage (Huang *et al.,* 2006)

UAF1 Protein Sequence:

MAAHHRQNTAGRRKVQVSYVIRDE
VEKYNRNGVNALQLDPALNRLFTAGRD
SIIRIWSVNQHKQDPYIASMEHHTDWVNDI
VLCCNGKTLISASSDTTVKVWNAHKGFCM
STLRTHKDYVKALAYAKDKELVASAGLDRQ
IFLWDVNTLTALTASNNTVTTSSLSGNKD
SIYSLAMNQLGTIIVSGSTEKVLRVWD
PRTCAKLMKLKGHTDNVKALLLNRDGTQ
CLSGSSDGTIRLWSLGQQRCIATYRVH
DEGVWALQVNDAFTHVYSGGRDRKIYCT
DLRNPDIRVLICEEKAPVLKMELDRSAD
PPPAIWVATTKSTVNKWTLKGIHN
FRASGDYDNDCTNPITPLCTQPDQVIKG
GASIIQCHILNDKRHILTKDTNNNVAY
WDVLKACKVEDLGKVDFEDEIKKRFKM
VYVPNWFSVDLKTGMLTITLDESDCFAAWV
SAKDAGFSSPDGSDPKLNLGGLLLQALLEY
WPRTHVNPMDEEENEVNHVNGEQENRVQK
GNGYFQVPPHTPVIFGEAGGRTLFRLL
CRDSGGETESMLLNETVPQWVIDITVDKN
MPKFNKIPFYLQPHASSGAKTLKKDRLSAS
DMLQVRKVMEHVYEKIINLDNESQTTSSSN
NEKPGEQEKEEDIAVLAEEKIELLCQDQV
LDPNMDLRTVKHFIWKSGGDLTLHYRQKST

UAF1 (regular text): Start ***bold italics*** (amino acid residues 1-677)
Accession number: NP_065890

Purification of the USP1/UAF1 heterodimer
To purify the USP1 [6His-tagged] / UAF1 [untagged] heterodimer the genes for these two proteins were co-expressed using an insect cell dual expression vector. Nickel resin capture was performed on the cell lysate derived from the lysed insect cells expressing the two proteins (Cohn *et al.,* 2007).

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