

# USP4 [6His-tagged]

## Deconjugating enzyme: Deubiquitylase

**Alternate Names:** Deubiquitinating enzyme 4, Ubiquitin carboxyl-terminal hydrolase 4, Ubiquitin-specific-processing protease 4, Ubiquitin thioesterase 4

**Cat. No.** 64-0001-050  
**Lot. No.** 30031

**Quantity:** 50 µg  
**Storage:** -70°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS



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 carboxyl-terminal hydrolase 4 (Ubiquitin Specific Protease 4; USP4) is a member of the cysteine protease enzyme family and cloning of the human gene was first described by Gupta *et al.* (1993). In 1995, USP4 was identified as a proto-oncogene related to USP6, showing a consistently elevated gene expression level in small cell tumours and lung adenocarcinomas suggesting that it may have a possible causative role in neoplasia (Gray *et al.*, 1995). USP4 has been implicated in a number of other processes, including protein quality control in the endoplasmic

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### Physical Characteristics

**Species:** human

**Source:** *E. coli* expression

**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:** ~111 kDa

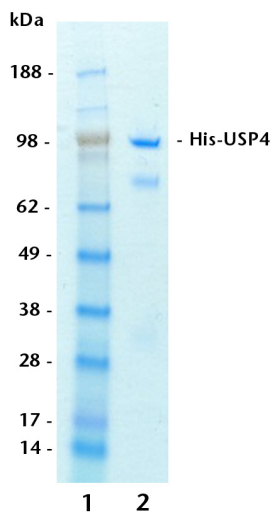
**Purity:** >76% by InstantBlue™ SDS-PAGE

**Stability/Storage:** 12 months at -70°C; aliquot as required

**Protein Sequence:** Please see page 2

### Quality Assurance

**Purity:**  
4-12% gradient SDS-PAGE  
InstantBlue™ staining  
Lane 1: MW markers  
Lane 2: 1 µg His-USP4



**Protein Identification:**  
Confirmed by mass spectrometry.

**Deubiquitylating Enzyme Assay:**  
The activity of His-USP4 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-USP4 was compared confirming the deubiquitylating activity of His-USP4.



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Lot-specific COA version tracker: v1.0.0

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### Background

Continued from page 1

reticulum and p53 and Wnt signalling. USP4 has also been reported to inhibit the kinase TAK1 that is ubiquitylated by the AKT regulator TRAF6 (Uras *et al.*, 2012).

#### References:

Gray DA, Inazawa J, Gupta K, Wong A, Ueda R and Takahashi T (1995) Elevated expression of Unph, a proto-oncogene at 3p21.3, in human lung tumors. *Oncogene* **10**, 2179-2183.

Gupta K, Copeland NG, Gilbert DJ, Jenkins NA and Gray DA (1993) Unp, a mouse gene related to the *trc* oncogene. *Oncogene* **8**, 2307-2310.

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 Rev Biochem* **78**, 363-397.

Uras IZ, List T and Nijman SM (2012) Ubiquitin-specific protease 4 inhibits mono-ubiquitination of the master growth factor signaling kinase PDK1. *PLoS one* **7**, e31003.

### Physical Characteristics

Continued from page 1

#### Protein Sequence:

**MGSSHHHHHSSGLEVLFQGP**GSMAEGGGCR  
ERPDAETQKSELGPLMRTTLQRGAQWYLID  
SRWFKQWKKYVGFDSWDMYNVGEHNLFP  
PIDNSGLFSDPESQTLKEHLIDELDYVLP  
TEAWNKLLNWyGCVegQqPIVRKVVEH  
GLFVKHCKVEVYLLLELKLcensDPTNVLSCH  
SKADTIATIEKEMRKLfNIPaerEtrL  
WNKYMSNTYEQLSKLDNTVQDAGLYQGQV  
LVIEPQNE DGTWPRQTLQSKSSTAPSRN  
FTTSPKSSASPYSSVSASLIANGDSTSTCG  
MHSSGVSRRGGSGFSASYNCOEPPSSHIQ  
PGLCGLGNLGNtCFMNSALQCLsNTAPLTDY  
FLKDEYEAEinRDnPLGMKGEIAEAYAE  
LIKQmWsgRDAHVAPRMFKTQVGRFAPQ  
FSGYQQQDSQELAFLLDGLHEDLN  
RVKKPYLElKdANGRPDAVVAKEAWEN  
HRLRNDsvIVDTFhGLfKstLVCPE  
CAKVSVTFDPFCYLTLPLPLKkDRVME  
VFLVPADPHCRPTQYRVTVPLMGAVSD  
LCEALSRLSGIAAENMVVADVYNHR  
FHKIFQmDEGLNHIMPRDIFVYEVC  
STSVdGSECvTLPVYFRERKSRPSST  
SSASALYgQPLLLSVPKHKLTLES  
LYQAVCDRISRYVKQPLPDEFgSSPLE  
PGACNGSRNSCEGEDEEMEhQEEGKE  
QLSETEGSGEDEPGNDPSETTQKKIK  
GQPCPKRLFTFLVNSYGTADINSLA  
ADGKLLKLNsrSTLAMDWdSETRRLY  
DEQeSEAYEKHvSMLQpQKKKkTtVAL  
RDCIElFTTMEtLGEHDPWYCPNCKK  
HQATKkFDLWslPKILVvHLKRFsYn  
RYWRDKLDtVVEFPiRGLNmSEfVc  
NLSARpyVYDLIAVSNHYGAMGVGH  
YTAYAKNKLNGKwYyFDDSNVSLAS  
EDQIVTKAAyVLFYQRrDDEFYkT  
PslSSSGSSDGGTRPSSSQGfGDDE  
ACsMDtN

Tag (**bold text**): N-terminal His  
Protease cleavage site: PreScission™ (LEVLFQ▼GP)  
USP4 (regular text): Start **bold italics** (amino acid residues 1-963)  
Accession number: NP\_003354



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