USP14 [6His-tagged]

Deconjugating enzyme

Alternate Names: Ubiquitin carboxyl-terminal hydrolase 14, Ubiquitin thioesterase 14, Ubiquitin-specific-processing protease 14

Cat. No.	**64-0018-050**	Quantity:	50 µg
Lot. No.	**30017**	Storage:	-70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

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 14 (USP14) is a member of the cysteine protease enzyme family and cloning of the gene was first described by Deshpande *et al.* (1996).

USP14 is a proteasome–associated DUB enzyme. Mammalian proteasomes are associated with three DUBs: USP14, UCHL5 (UCH37) and RPN11 (POH1). UCHL5 and USP14 reside on the 19S regulatory particle and remove ubiquitin from the substrate before substrate degradation whereas RPN11's activity is delayed until the proteasome is committed to degrading the substrate (Lee *et al.*, 2010). Ubiquitin-tagged substrates

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Physical Characteristics

Species: human

Source: *E. coli*

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: ~58.5 kDa

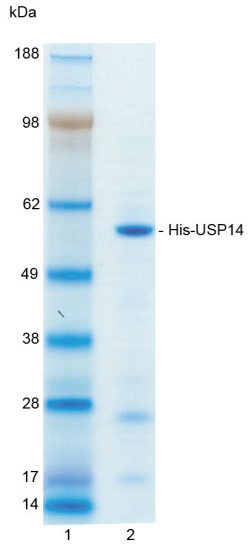
Purity: >60% by InstantBlue™ SDS-PAGE

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-USP14



Protein Identification:
Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay: His-USP14 has limited activity against the fluorogenic substrate Ubiquitin-Rhodamine110-Glycine. The DUB activity of His-USP14 is known to be potentiated by proteasomes. See Cat# 64-1010-096 for the dual product (USP14 & 26S proteasome [Ub-VS-treated]) proteasome-activated USP14.

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Lot-specific COA version tracker: v1.0.0

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Background

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are degraded by the 26S proteasome, which is a multi-subunit complex comprising a proteolytic 20S core particle capped by 19S regulatory particles (Wang *et al.*, 2007). The approval of bortezomib for the treatment of multiple myeloma validated the 20S core particle as an anticancer drug target. Recent experiments have now shown that the 19S regulatory particle is also a potential anticancer drug target (D'Arcy *et al.*, 2011). Alterations in USP14 can lead to proteasome dysfunction and neurological disease. Recent in vitro experiments show that USP14 can also stabilize the expression of over-expressed, disease-associated proteins such as tau and ataxin-3 (Jin *et al.*, 2012). A number of other studies over the years have implicated USP14 in cancer. Ishiwata *et al.*, (2001) first found that the USP14 expression was upregulated in leukemic cells, Shinji *et al.*, (2006) found that the USP14 expression in colorectal cancer is associated with liver and lymph node metastases and Chuensumran, *et al.*, (2011) that USP14 expression is associated with intrahepatic cholangiocarcinoma cell differentiation. A recent review by Wu *et al.* (2013) also reports that USP14 is a tumour-promoting factor and a promising therapeutic target for non-small cell lung cancer (NSCLC).

The DUB activity of USP14 is known to be activated by proteasomes and alone USP14 has little DUB activity and as such may be used as a control in experiments alongside those performed with proteasome-activated USP14: Such experiments are typically performed using a proteasome preparation where the native DUBs – including USP14 – have been removed and/or inactivated before the proteasome preparation is added back to recombinantly expressed USP14.

References:

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Deshpande KL, Seubert PH, Tillman DM, Farkas WR and Katze JR (1996) Cloning and characterization of cDNA encoding the rabbit tRNA-guanine transglycosylase 60-kilodalton subunit. *Arch Biochem Biophys* **326**, 1-7.

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Lee BH, Lee MJ, Park S, Oh DC, Elsasser S, Chen PC, et al. (2010) Enhancement of proteasome activity by a small-molecule inhibitor of USP14. *Nature* **467**, 179-184.

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Physical Characteristics

Continued from page 1

Protein Sequence:

MGSSHHHHHHSSGLEVLFQGPGS***M***PLYS
VTVKWGKEKFEGVELNTDEPPMVFKAQL
FALTGVQPARQKVMVKGGTLKDDDWGNI
KIKNGMTLLMMGSADALPEEPSAKTVFVED
MTEEQLASAMELPCGLTNLGNTCYMNAT
VQCIRSVPELKDALKRYAGALRASGE
MASAQYITAALRDLFDSMDKTSSSIPPI
ILLQFLHMAFPQFAEKGEQGQYLQQDAN
ECWIQMMRVLQQKLEAIEDDSVKETDSS
SASAATPSKKKSLIDQFFGVEFETTMKCTE
SEEEEVTKGKENQLQLSCFINQEVKYLFT
GLKLRLQEEITKQSPTLQRNALYIKSSKISR
LPAYLTIQMVRFFYKEKESVNAKVLKDVK
FPLMLDMYELCTPELQEKMVSFRSKFK
DLEDKKVNQQPNTSDKKSSPQKEVKYEPFS
FADDIGSNNCGYYDLQAVLTHQGRSSSS
GHYVSWVKRKQDEWIKFDDDKVSIVTPEDIL
RLSGGGDWHIAYVLLYGPRRVEIMEEESEQ

Tag (**bold text**): N-terminal 6His
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
USP14 (regular text): Start ***bold italics*** (amino acid residues 1-494)
Accession number: AAH03556

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