USP14 & 26S Proteasome [Ub-VS treated]

Deconjugating enzyme

Cat. No. **64-1010-096**
Lot. No. **30198**

Quantity: 96 assay points
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).

The ubiquitin–proteasome system (UPS) targets selected proteins for degradation by the 26S proteasome. The initial steps in this pathway generate proteins that are covalently tagged with a polyubiquitin chain that is then recognized by ubiquitin receptors of the 26S proteasome. This is a large

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Physical Characteristics

Species: human

Source: USP14: *E.coli*
26S Proteasome: Transformed HEK293 cells using Lan Huang's cell line and extraction protocol (Wang *et al.,* 2007)

Quantity: 96 assay points

Concentration: n/a

Formulation: DTT containing buffer

Molecular Weight: USP14: ~58.5 kDa

Purity: n/a

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

Protein Sequences: Please see page 2

Quality Assurance

Protein Identification:
USP14 confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay: The activity of His-USP14 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 with USP14 in the presence or absence of 26S proteasome [Ub-VS] was compared confirming the deubiquitylating activity of 26S proteasome [Ub-VS]-activated His-USP14. See Cat. No. 64-0018-050 for the unactivated version of this enzyme which has limited Ubiquitin-Rhodamine110-Glycine activity.

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Lot-specific COA version tracker: v1.0.0

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complex composed of a 20S catalytic core particle and two 19S regulatory particles (Kok, *et al.*, 1993) that catalyse the final step in the pathway. While the 20S particle is composed of a catalytic chamber for protein degradation, collectively the proteins that comprise the 19S particle perform several proteasomal functions that include recognition of ubiquitylated substrates, cleavage of the polyubiquitin chain for ubiquitin recycling, control of access to the 20S proteolytic chamber, and substrate unfolding and subsequent translocation into the 20S core particle for degradation (Boehringer, *et al.*, 2012). Mammalian proteasomes are associated with three DUBs: USP14, UCHL5 (UCH37) and RPN11 (POH1). UCHL5 and USP14 reside on the 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). The DUB activity of USP14 is known to be activated by proteasomes.

The 26S proteasome preparation in this product was prepared using the same protocol as described in Wang *et al.* (2007). The 26S proteasome DUB activity was removed through washing and treatment with ubiquitin–vinylsulphone (Ub–VS) which forms an adduct with the active site cysteine in DUBs of the thiol protease class (Lee, *et al.*, 2010).

This product comprises an optimised molar ratio of 20nM USP14:1.25nM 26S proteasome [Ub-VS] based on assuming a 2.5MDa molecular weight for the 26S proteasome in accordance with Wang *et al.* (2007).

References:

Boehringer J *et al.* (2012) Structural and functional characterization of Rpn12 identifies residues required for Rpn10 proteasome incorporation, *Biochem J* **448**, 55-65.

Deshpande KL *et al.* (1996) Cloning and characterization of cDNA encoding the rabbit tRNA-guanine transglycosylase 60-kilodalton subunit, *Arch Biochemistry Biophys* **326**, 1-7.

Kok K *et al.* (1993) A gene in the chromosomal region 3p21 with greatly reduced expression in lung cancer is similar to the gene for ubiquitin-activating enzyme, *Proc Natl Acad Sci U S A* **90**, 6071-6075.

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.

Lee BH *et al.* (2010) Enhancement of proteasome activity by a small-molecule inhibitor of USP14, *Nature* **467**, 179-184.

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

Wang X *et al.* (2007) Mass spectrometric characterization of the affinity-purified human 26S proteasome complex, *Biochemistry* **46**, 3553-3565.

Physical Characteristics

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Protein Sequence: USP14

MGSSHHHHHHSSGLEVLFQGPGS***M***PLYS
VTVKWGKEKFEGVELNTDEPPMVFKAQL
FALTGVQPARQKVMVKGGTLKDDDWGNI
KIKNGMTLLMMGSADALPEEPSAKTVFVED
MTEEQLASAMELPCGLTNLGNTCYMNAT
VQCIRSVPELKDALKRYAGALRASGE
MASAQYITAALRDLFDSMDKTSSSIPPI
ILLQFLHMAFPQFAEKGEQGQQYLQQDAN
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

Protein Sequence: 26S Proteasome

The 26S proteasome preparation in this product was prepared using the same protocol as described in Wang *et al.* (2007). Please refer to Wang *et al.* (2007) for a breakdown of the proteasome subunits and other components identified in such a purified complex.

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