26S Proteasome [Ub-VS treated]

Proteasome

Cat. No. **65-1020-010**
Lot. No. **30189**

Quantity: 10 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

Background

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 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

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Physical Characteristics

Species: human

Source: transformed HEK293 cells

Quantity: 10 µg

Concentration: 0.2 mg/ml

Formulation: 50 mM Tris/HCl pH7.4, 10% glycerol, 1 mM ATP

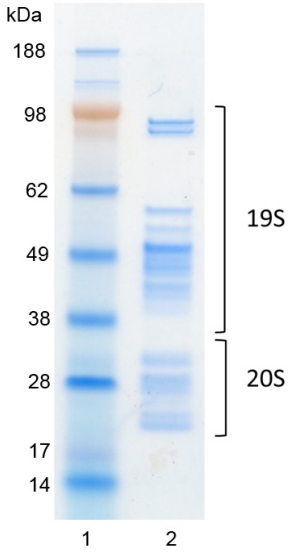
Molecular Weight: ~2500 kDa

Stability/Storage: 12 months at -70˚C; avoid multiple freeze/thaw cycles

Protein Sequence:
The 26S proteasome preparation 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 from the purified complex. The 26S proteasome-associated 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).

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 3 µg 26S Proteasome [Ub-VS treated]



Protein Identification:
The 26S proteasome preparation was prepared using the same protocol as described in Wang *et al.* (2007). Upon separation by 1D SDS-PAGE, the overall gel protein staining pattern of the purified 26S proteasome complex was visually and qualitatively very similar to that reported in Wang *et al.* (2007), Figure 1D.

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Lot-specific COA version tracker: v1.0.0

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References:

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

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 USA* **90**, 6071-6075.

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

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

Quality Assurance

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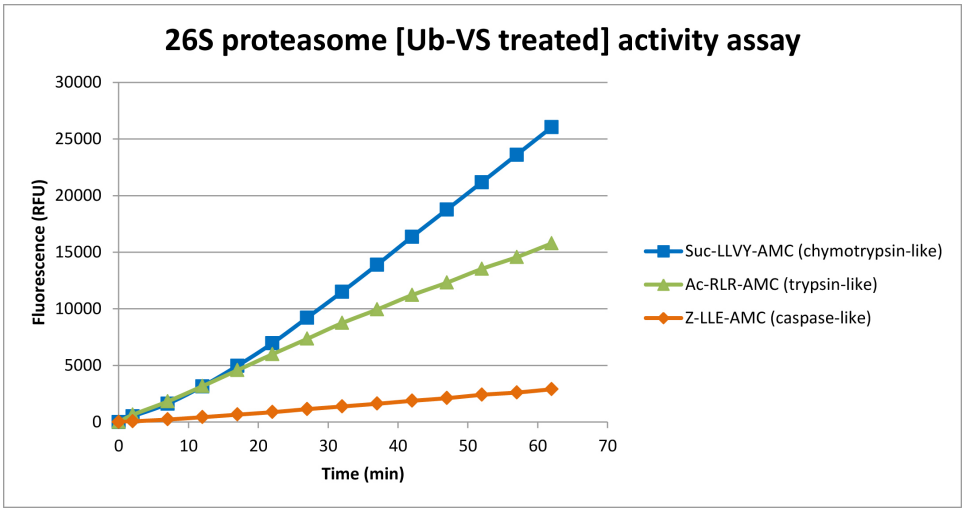
26S Proteasome Activity Assay:
26S proteasome proteolytic activity was validated by determining the increase in fluorescence measured as a result of the enzyme catalysed cleavage of fluorogenic peptide substrates (Suc-LLVY-AMC, Ac-RLR-AMC and Z-LLE-AMC) generating the peptide and AMC. Incubation of the substrate in the presence or absence of 26S proteasome was compared confirming the proteolytic activity of the 26S proteasome.



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Lot-specific COA version tracker: v1.0.0