USP14 Activation Kit

Cat. No. **67-0014-001**
Lot. No. **30388**

Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

Kit Utility

The USP14 Activation Kit contains USP14 [6His-tagged] and 26S Proteasome [Ubiquitin-Vinyl Sulfone (Ub-VS) treated] plus sufficient 5x DUB Assay Buffer for preparing the assays. Through the addition of your own DUB substrate of interest, for example Ubiquitin-Rhodamine 110 (Cat# 60-0117-050), you can determine the optimal ratio of USP14:26S Proteasome [Ub-VS treated] appropriate for your assay. (Note: USP14 has no detectable catalytic activity in the absence of activation by the Ub-VS treated 26S Proteasome preparation provided. See Background for more information.)

Once your preferred USP14 activation ratio has been determined, an application of the USP14 Activation Kit that might be of greatest interest is to test for inhibitors of the interaction of USP14 with the proteasome. Experiments may include pre-incubating the test compound(s) with USP14 and/or the 26S Proteasome [Ub-VS treated] prior to mixing.

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

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Components

Product	Amount	Cat. No.
USP14 [6His-tagged]	5 µg	64-0018-005
26S Proteasome [Ub-VS treated]	10 µg	65-1020-010
5x DUB Assay Buffer	0.5 ml	64-2001-500

For further information on the deconjugating enzyme or the 26S Proteasome supplied in this kit please refer to the Ubiquigent website: www.ubiquigent.com.

Physical Characteristics

USP14 [6His-tagged]

Species: human

Source: *E. coli*

Quantity: 5 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: >56% by InstantBlue™ SDS-PAGE

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

26S Proteasome [Ub-VS treated]

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.

Protocol

For guidance: The ratio used in Ubiquigent's pre-formulated product USP14+26S Proteasome [Ub-VS-treated] (Cat# 64-1010-096) is 20 nM USP14:1.25 nM 26S Proteasome [Ub-VS treated]. Lee *et al.* (2010) used 4 nM USP14:1 nM 26S Proteasome [Ub-VS treated].

1. Prepare sufficient 1x DUB Assay Buffer using the 5x DUB Assay Buffer stock (Cat# 64-2001-500).
2. Prepare a 20 µl reaction per well using the enzyme and proteasome reagents provided, your test compound (if required) and your substrate of choice made up to 20 µl with 1x DUB Assay Buffer.
3. Incubate for 40 min at room temperature.
4. Analyse samples appropriately according to substrate choice.

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Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

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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 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 through it's interaction with the proteasome complex.

The 26S proteasome product in this kit 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).

References:

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

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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.

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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.

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