Activated USP14 DUB Assay Kit

Cat. No. **67-0015-096**
Lot. No. **30205**

Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

Kit Utility

The Activated USP14 DUB Assay Kit contains all the reagents required to perform a USP14 DUB assay. The kit comprises Ubiquigent's pre-formulated product USP14 & 26S Proteasome [Ubiquitin-Vinyl Sulfone (Ub-VS) treated] (Cat# 64-1010-096) at an optimised molar ratio ready for use in a USP14 deubiquitylase assay. This kit also contains sufficient 5x DUB Assay Buffer (Cat# 64-2001-500) for preparing the assays as well as 4x Ubiquitin-Rhodamine 110 substrate (Cat# 60-0122-500) and 5x DUB Assay Stop Buffer (Cat# 64-2002-500).

An application of the Activated USP14 DUB Assay Kit that might be of greatest interest is to test for inhibitors of 'proteasome-activated'-USP14.

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

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Components

Product	Amount	Cat. No.
USP14 & 26S Proteasome [Ub-VS treated]	96 assay tests	64-1010-096
5x DUB Assay Buffer	500 µl	64-2001-500
4x Ubiquitin-Rhodamine 110	500 µl	60-0122-500
5x DUB Assay Stop Buffer	500 µl	64-2002-500

For further information on the products supplied in this kit please refer to the Ubiquigent website: www.ubiquigent.com.

Physical Characteristics

USP14 & 26S Proteasome [Ub-VS treated]

This product is supplied as a 4x concentrated stock. The final assay concentration provides an optimised molar ratio of 20 nM USP14:1.25 nM 26S proteasome [Ub-VS] based on assuming a 2.5 MDa molecular weight for the 26S proteasome in accordance with Wang *et al.* (2007).

Species: human

Source: USP14 = *E.coli* 26S Proteasome = Transformed HEK293 cells

Quantity: 96 assay tests

Formulation: DTT containing buffer

Molecular Weight: USP14 = ~58.5 kDa; 26S Proteasome = ~2500 kDa

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

Protocol

1. With the addition of H_2O, prepare sufficient 1x DUB Assay Buffer using the 5x DUB Assay Buffer stock (Cat# 64-2001-500).

2. For a 20 µl reaction volume, pipette 5 µl/well of USP14 & 26S proteasome [Ub-VS treated] (Cat# 64-1010-096).

3. Pipette 10 µl/well of your test compound (or 1x DUB Assay Buffer) at 2x concentrated stock. Alternatively you may wish to deliver your test compound in a smaller volume in which case split the 10 µl volume between compound in 1x DUB Assay Buffer and 1x DUB Assay Buffer. Further you may wish to deliver the compound in DMSO. The assay

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Lot-specific COA version tracker: v1.0.0

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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 enzymes, 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 its 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.

Deshpande KL *et al.* (1996) Cloning and characterization of cDNA encoding the rabbit tRNA-guanine transglycosylase 60-kilodalton subunit, *Arch Biochem 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 USA* **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.

Protocol

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may be operated at a final concentration of 1% DMSO (the assay *may* be tolerant to higher DMSO concentrations; to be tested empirically by the user).

4. Pre-incubate enzyme with compound for 15 min at room temperature.
5. Pipette 5 µl/well of 4x Ubiquitin-Rhodamine 110 (Cat# 60-0122-500).
6. Incubate for 40 min at room temp.
7. Pipette 5 µl of 5x DUB Assay Stop Buffer (Cat# 64-2002-500).
8. Read the fluorescence intensity in a microplate reader (excitation 485 nm, emission 535 nm).

Control reactions: Ubiquigent recommends running two control reactions;

a) containing no test compound (plus enzyme control = 100% enzyme activity), and,

b) containing no test compound or enzyme (no enzyme control = 0% enzyme activity).

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