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Zuschläge

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Description

The Ubiquitin specific peptidase 14 (USP14) Inhibitor Screening Assay Kit is a 96-well fluorogenic assay designed to measure the activity of the deubiquitinating (DUB) enzyme USP14 for screening and profiling applications. The kit contains enough purified USP14 protein, Ubiquitinated-AMC substrate, and assay buffer for 100 reactions.

To determine the effect of an inhibitor on USP14 activity, the enzyme should be preincubated with or without the test inhibitor prior to adding the Ub-AMC substrate to the reaction. The assay was functionally validated using Ub-Aldehyde, a potent inhibitor of DUB subfamilies Ubiquitin C-terminal Hydrolases (UCHs), Ubiquitin-Specific Proteases (USPs), Ovarian Tumor Proteases (OTU), and Machado-Josephin Domain (MJD) proteases.

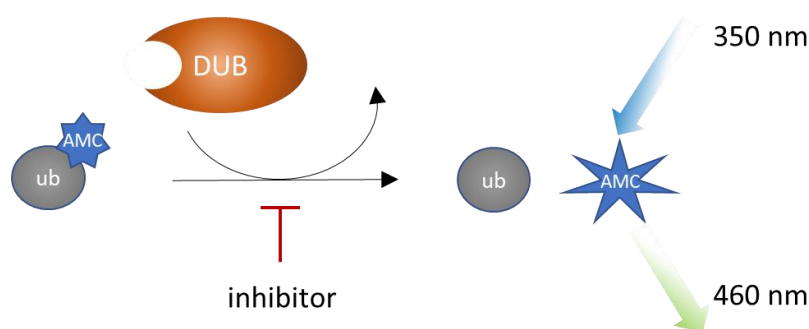


Figure 1: Illustration of the assay principle.

Ubiquitin-AMC is a fluorogenic substrate for ubiquitin hydrolases based on the C-terminus derivatization of ubiquitin with 7-amido-4-methylcoumarin (AMC). In conjugated form, the energy emitted from the fluorochrome AMC is quenched. Upon proteolysis, AMC is no longer quenched and emits fluorescence with excitation/emission maxima of 350/460 nm. The increase in fluorescence is proportional to the DUB activity.

Background

Ubiquitin specific peptidase 14 (USP14) belongs to a large group of ubiquitin-specific proteases capable of cleaving ubiquitin from other proteins. These enzymes are also referred to as deubiquitinating peptidases, deubiquitinases (DUBs), ubiquitin proteases, ubiquitin hydrolases or ubiquitin isopeptidases. They contribute to the ubiquitin signaling pathway by countering the signal induced by ubiquitin conjugating enzymes and ligases. DUBs are new therapeutic targets for neurodegenerative diseases, cancer, diabetes, and autoimmune pathologies.

Applications

Enzyme kinetics and screening small molecule inhibitors for drug discovery and High-Throughput Screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
80364	USP14, FLAG-Tag*	8 µg	-80°C
81150	Ub-AMC Substrate	5 µl	-80°C
79274	10x PR-01 Assay Buffer	3 x 1 ml	-80°C
	0.5 M DTT	200 µl	-80°C
79685	96-well black microplate	1	Room Temp

* The concentration of protein is lot-specific and will be indicated on the tube containing the protein.

Materials Required but Not Supplied

- Adjustable micropipettor and sterile tips
- Plate reader

Stability



This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety



This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include a “Negative control”, “Positive control” and “Test inhibitor”.
- If the assay plate is going to be used more than once, prepare enough of each reagent for this portion of the assay and aliquot the remaining undiluted reagents into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C or as recommended for each reagent.
Unused diluted proteins should be discarded.



Protect Ub-AMC from direct exposure to light.

1. Thaw **10x PR-01 Assay Buffer** and **0.5 M DTT**.
2. Dilute 0.5 M DTT 100-fold in 10x PR-01 to reach a 5 mM DTT solution.
3. Prepare a 10-fold dilution of 10x PR-01 Assay Buffer (containing DTT) in distilled water. This makes **1x Assay Buffer**. Discard the unused 1x Assay buffer at the end of the day.
4. Thaw **USP14** on ice. Briefly spin the tube to recover its full content.
5. Dilute USP14 to 3.2 ng/μl in 1x Assay Buffer (you need 25 μl/well).
Keep the diluted protein on ice until use. Do not freeze and re-use the diluted protein.
6. Add 25 μl of diluted USP14 to all wells except “Negative control”.
7. Add 25 μl of 1x Assay Buffer to the “Negative Control” wells.
8. Prepare the Test inhibitor (5 μl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 μl.
 - 8.1. If the Test Inhibitor is water-soluble, prepare serial dilutions in 1x Assay Buffer at concentrations 10-fold higher than the desired final concentrations.

OR

8.2. If the Test inhibitor is soluble in DMSO, prepare the test inhibitor in 100% DMSO at a concentration 100-fold higher than the highest desired final concentration, then dilute the inhibitor 10-fold in 1x Assay Buffer to prepare the highest concentration of the serial dilutions. The concentration of DMSO is now 10%.

Using the 1x Assay Buffer containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

For positive and negative controls, prepare 10% DMSO in Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO in the assay should not exceed 1%.

9. Add 5 μ l of Test inhibitor to each well designated "Test Inhibitor".
10. Add 5 μ l of Diluent Solution to the "Positive Control" and "Negative Control" wells.
11. Preincubate the Test inhibitor with the diluted USP14 for 30 minutes at Room Temperature with gentle agitation.
12. Dilute **Ub-AMC Substrate** 400-fold in 1x Assay Buffer.
13. Initiate the reaction by adding 20 μ l of diluted Ub-AMC Substrate to all wells. **Protect your samples from direct exposure to light** and incubate at Room Temperature for 30 minutes.

Component	Negative control	Positive Control	Test Inhibitor
1x Assay Buffer	25 μ l	-	-
USP14 (3.2 ng/ μ l)	-	25 μ l	25 μ l
Test inhibitor	-	-	5 μ l
Diluent Solution	5 μ l	5 μ l	-
30 minutes at Room Temperature			
Ub-AMC Substrate	20 μ l	20 μ l	20 μ l
30 minutes at Room Temperature			
Total	50 μl	50 μl	50 μl

14. Read the fluorescence intensity of the samples (lexcitation = 350 nm; lemission = 460 nm) in a fluorescence reader.

Example Results

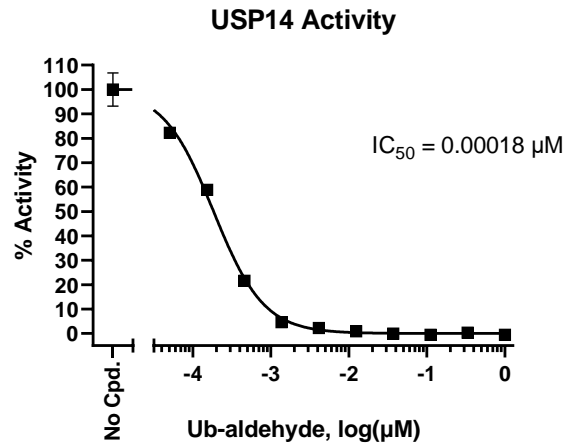


Figure 2. USP14 activity is inhibited by Ub-Aldehyde.

USP14 activity was measured in the presence of increasing concentrations of Ub-Aldehyde (South Bay Bio #PS0031). Results are expressed as percentage of activity relative to the positive control (measured in the absence of inhibitor and set at 100%).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

Related Products

Products	Catalog #	Size
USP14, Flag-Tag Recombinant	80364	50 μg
USP1 Inhibitor Screening Assay Kit	78831	96 reactions
USP5 Inhibitor Screening Assay Kit	78832	96 reactions
USP20 Inhibitor Screening Assay Kit	78840	96 reactions
UCHL1 Inhibitor Screening Assay Kit	78833	96 reactions