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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The UCHL3 Inhibitor Screening Assay Kit is a 96-well format fluorogenic assay designed to measure the activity of the deubiquitinating (DUB) enzyme UCHL3 for screening and profiling applications. The kit contains enough purified UCHL3 protein, Ubiquitinated-AMC substrate and assay buffer for 100 reactions.

To determine the effect of an inhibitor on UCHL3 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.

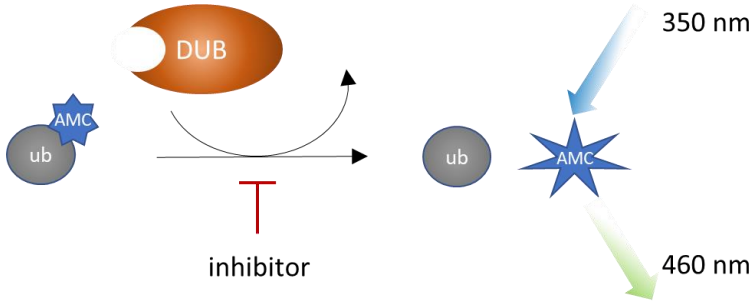

Figure 1: Illustration of the assay principle.

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

Background

Ubiquitin carboxyl-terminal hydrolase isozyme L3 (UCHL3, also known as Ubiquitin thioesterase L3), 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 remove the covalently bound ubiquitin and contribute to the ubiquitin signaling pathway by countering the signal induced by ubiquitin conjugating enzymes and ligases. DUBs are a new therapeutic target for neurodegenerative diseases, cancer, diabetes, and autoimmune pathologies.

Applications

Enzyme kinetics and screening small molecule inhibitors for drug discovery and high-throughput applications.

Supplied Materials

Catalog #	Name	Amount	Storage
80353	UCHL3, His-Tag*	≥1 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
Fluorescent 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 duplicates.
- The assay should include a "Negative control", a "Positive control," and a "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**. Dilute 0.5 M DTT 100-fold in 10x PR-01 Assay Buffer to reach a 5 mM DTT solution. Store excess buffer in aliquots at -20°C. Do not freeze-thaw the aliquots more than once.

2. Prepare a 10-fold dilution of 10x PR-01 Assay Buffer (containing DTT) in distilled water to create **1x Assay Buffer**. Discard the unused 1x Assay Buffer at the end of the day.

3. Thaw **UCHL3** on ice. Briefly spin the tube to recover its full content.

4. Dilute **UCHL3** to 0.4 pg/µl in Assay Buffer (you need 25 µl/well).

   Keep the diluted protein on ice until use. Do not freeze and re-use the diluted protein.

5. Add 25 µl of diluted UCHL3 to all wells except "Negative control" wells.

6. For the "Negative Control" add 25 µl of 1x Assay Buffer.

7. 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.

   7.1. If the Test Inhibitor is water-soluble, prepare serial dilutions in the 1x Assay Buffer at concentrations 10-fold higher than the desired final concentrations. The 1x Assay Buffer is the Diluent Solution.
   OR
   7.2. 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 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 1x 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%.

8. Add 5 µl of Test inhibitor to each well designated "Test Inhibitor".

9. Add 5 µl of Diluent Solution to the "Positive Control" and "Negative Control" wells.

10. Preincubate the Test inhibitor with the diluted UCHL3 for 30 minutes at Room Temperature with gentle agitation.

11. Dilute **Ub-AMC Substrate** 400-fold in 1x Assay Buffer.

12. Initiate the reaction by adding 20 µl 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 µl	-	-
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
UCHL3 (0.4 pg/µl)	-	25 µl	25 µl
30 minutes at room temperature			
Ub-AMC Substrate	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

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

Example Results

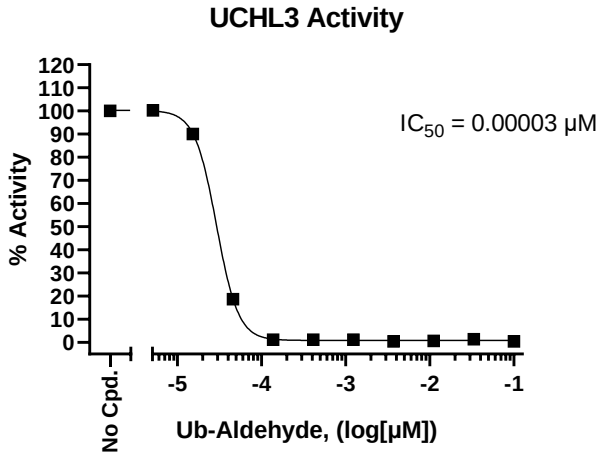

Figure 2. UCHL3 activity is inhibited by Ub-Aldehyde.
UCHL3 activity was measured in the presence of increasing concentrations of Ub-Aldehyde (South Bay Bio #PS0031). Results are expressed as percentage of activity relative to positive control (measured in the absence of inhibitor and set at 100%).

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 further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
USP1 Inhibitor Screening Assay Kit	78831	96 reactions
USP5 Inhibitor Screening Assay Kit	78832	96 reactions
USP7 Inhibitor Screening Assay Kit	79256	96 reactions
USP20 Inhibitor Screening Assay Kit	78840	96 reactions