Description

The USP13 Inhibitor Screening Assay Kit is a 96-well format fluorogenic assay designed to measure the activity of the deubiquitinating (DUB) enzyme USP13 (ubiquitin specific peptidase 13) for screening and profiling applications. The kit contains enough purified USP13 protein, Ubiquitinated-AMC substrate, and assay buffer for 100 reactions.

To determine the effect of an inhibitor on USP13 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 proteases (MJD).

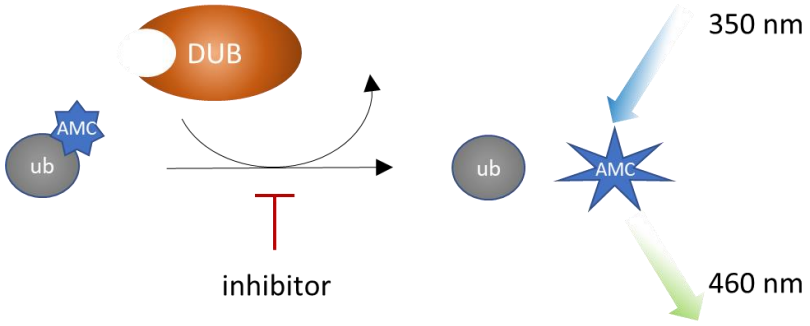

Figure 1: Illustration of the assay principle.

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 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

USP13 (ubiquitin carboxyl-terminal hydrolase 13), also known as isopeptidase T 3 (ISOT-3) is a DUB (deubiquitination) enzyme. It participates in multiple crucial cellular processes, such as autophagy, DNA damage response (DDR), and ERAD (endoplasmic reticulum-associated degradation) by deubiquitinating proteins involved in those functions. It also plays a role in diseases such as cancer and neurodegeneration. It can play both a suppressor and activator role in tumorigenesis. For instance, low levels of USP13 correlate with low levels of PTEN (phosphatase and tensin homolog), as UPS13 stabilizes the protein. The development of USP13 inhibitors has been of interest, however due to the similarity between USP13 and USP10 can be hard to accomplish. A deeper understanding of all its cellular roles and how to best target its function in a specific context may prove beneficial in cancer therapy.

Applications

Enzyme kinetics studies and screening small molecule inhibitors for drug discovery and high-throughput screening (HTS) applications.

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Supplied Materials

Catalog #	Name	Amount	Storage
102018	USP13, His-Tag, FLAG-Tag*	50 µg	-80°C
81150	Ub-AMC Substrate	5 µl	-80°C
79274	10x PR-01 Assay Buffer	3 x 1 ml	-80°C
82735	0.5 M DTT	200 µl	-20°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
- Fluorimeter capable of excitation at λ=350-380 nm and detection at λ=440-460 nm

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.

Contraindications

- The final concentration of DMSO in the assay should not exceed 1%.
- Compounds that are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone is tested to determine any potential interference of the compound with the assay results.

Assay Protocol

- All samples and controls should be performed in duplicates.
- The assay should include "Negative Control", "Positive Control," and a "Test Inhibitor" conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using Ub-Aldehyde as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC50 value shown in the validation data below.

Protect Ub-AMC from direct exposure to light.

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1. Thaw **10x PR 01 Assay Buffer** and **0.5 M DTT**.

2. Dilute **0.5 M DTT** 100-fold with **10x PR-01 Assay Buffer** to reach a 5 mM DTT solution.

   Note: Store excess solution in aliquots at -20°C. Do not freeze-thaw the aliquots more than once.

3. Prepare a 10-fold dilution of 10x PR-01 Assay Buffer (containing DTT) in distilled water. This makes **1x Assay Buffer.**

   Note: Discard unused 1x Assay Buffer at the end of the day.

4. Thaw **USP13** on ice. Briefly spin the tube to recover its full content.

5. Dilute USP13 to 20 ng/µl with 1x Assay Buffer (you need 25 µl/well).

6. Add 25 µl of diluted USP13 to all wells, except "Negative Control" wells.

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

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 the 1x Assay Buffer at concentrations 10-fold higher than the desired final concentrations.

   For the positive and negative controls, use 1x Assay Buffer (Diluent Solution).

   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 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%.

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

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

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11. Preincubate the Test inhibitor with the diluted USP13 for 30 minutes at Room Temperature (RT) with gentle agitation.

12. Dilute **Ub-AMC Substrate** 400-fold with 1x Assay Buffer.

13. Initiate the reaction by adding 20 µl of **diluted Ub-AMC Substrate** to all wells.

Protect your samples from direct exposure to light.

14. Incubate at RT for 1 hour.

Component	Negative Control	Positive Control	Test Inhibitor
1x Assay Buffer	25 µl	-	-
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted USP13 (20 ng/µl)	-	25 µl	25 µl
30 minutes at Room Temperature			
Diluted Ub-AMC Substrate	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

15. Read the fluorescence intensity of the samples (λexcitation = 350-380 nm; λemission = 440-460 nm) in a fluorescence reader.

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Example Results

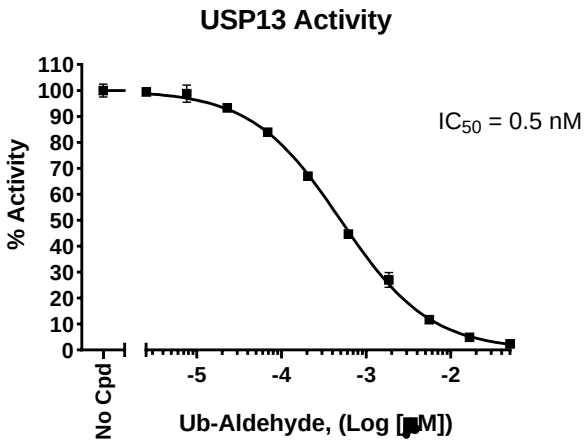

Figure 2. USP13 activity is inhibited by Ub-Aldehyde.
USP13 activity was measured in the presence of increasing concentrations of Ub-Aldehyde (South Bay Bio #PS0031). Results are expressed as percentage of activity relative to the positive control (measured in the absence of inhibitor and set at 100%).

Data shown is representative.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

References
Liao X., *et al.*, 2022 *Front. Cell Dev. Biol.* 10:3389.

Related Products

Products	*Catalog #*	*Size*
USP21 Inhibitor Screening Assay Kit	82944	96 reactions
USP21, His-SUMO-tags Recombinant	81101	25 µg
USP22 Inhibitor Screening Assay Kit	82742	96 reactions
USP15 Inhibitor Screening Assay Kit	82940	96 reactions
DUB-Freedom™ Inhibitor Screening Assay Kit	78895	96 reactions

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