Description

The USP8 Inhibitor Screening Assay Kit is a fluorogenic assay designed to measure the activity of the deubiquitinating (DUB) enzyme USP8 (ubiquitin specific peptidase 8) for screening and profiling applications. The kit comes in a convenient 96-well format and contains enough purified recombinant human USP8 protein, Ubiquitinated-AMC substrate, and assay buffer for 100 reactions.

To determine the effect of an inhibitor on USP8 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.

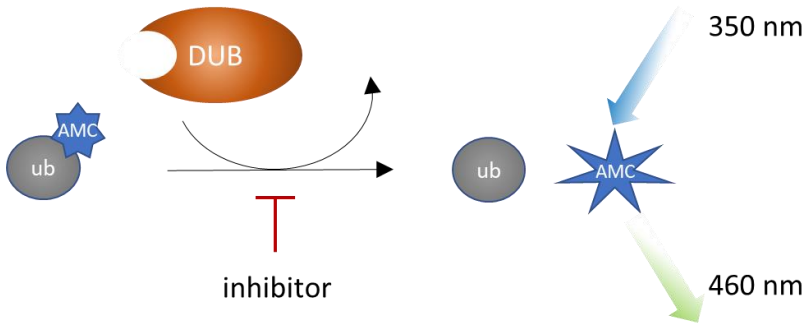

Figure 1: Illustration of the assay principle.

Ubiquitin-AMC (Ub-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 the fluorochrome AMC is quenched. Upon release from Ub, AMC emissions are no longer quenched fluorescence with λexcitation/λemission maxima of 350/460 nm is emitted. The increase in fluorescence is proportional to the DUB activity.

Background

Ubiquitin specific peptidase 8 (USP8), 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, ubiquitin isopeptidases. They contribute to the ubiquitin signaling pathway by countering the signal induced by ubiquitin conjugases and ligases. USP8 is involved in protein endosomal sorting and has been linked to several cancer types (lung, cervical, breast cancer and hepatocellular carcinoma). USP8 promotes the development of drug resistance in prostate cancer (PCa), with knockdown of USP8 resulting in docetaxel being more effective. In addition, its knockdown it suppresses EGFR (epidermal growth factor receptor), PI3K (phosphoinositide 3-kinase) and NF-κB (nuclear factor kappa-light chain enhancer of activated B cells) pathways. Further studies into the function of USP8 and the discovery of new inhibitors will open avenues of treatment for diseases like PCa.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
80358	USP8 FLAG-Tag*	3 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 "Negative Control", "Positive Control" and "Test inhibitor" conditions.
- 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 5 mM DTT with distilled water to create **1x Assay Buffer**. Discard the unused 1x Assay Buffer at the end of the day.

4. Thaw **USP8** on ice. Briefly spin the tube to recover its full content.

5. Dilute USP8 to 1.2 ng/µl in 1x Assay Buffer (you need 25 µl/well).

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

6. Add 25 µl of diluted USP8 to all wells except "Negative Control" wells.

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. 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%.

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.

11. Preincubate the Test inhibitor with the diluted USP8 for 30 minutes at Room Temperature (RT) with gentle agitation.

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

13. Add 20 µl of diluted Ub-AMC Substrate to all wells. **Protect your samples from direct exposure to light.**

14. Incubate at RT for 30 minutes.

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

Component	Negative control	Positive Control	Test Inhibitor
1x Assay Buffer	25 µl	-	-
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted USP8 (1.2 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**

Example Results

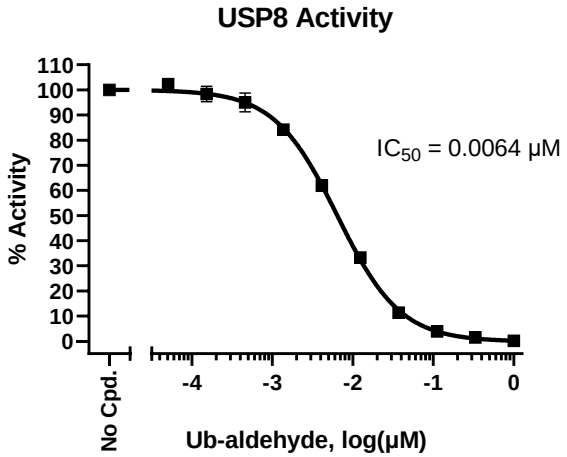

Figure 2. USP8 inhibition by Ub-Aldehyde.
USP8 activity was measured in the presence of increasing concentrations of Ub-Aldehyde (South Bay Bio #PS0031). Results are expressed as percent activity, in which the activity of USP8 in absence of inhibitor is set to 100%.

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*
DUB-Freedom™ Inhibitor Screening Assay Kit	78895	96 reactions
ChooseE2-Opti™ Intrachain TR-FRET Assay Kit	78561	384 reactions
ChooseE3- Opti™ Intrachain TR-FRET Assay Kit	78560	384 reactions