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Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
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Description

The USP11 Inhibitor Screening Assay Kit is a 96-well format fluorogenic assay designed to measure the activity of the deubiquitinating (DUB) enzyme USP11 (ubiquitin specific peptidase 11) for screening and profiling applications. The kit contains enough purified USP11 protein, Ubiquitinated-AMC substrate, and assay buffer for 100 reactions.

To determine the effect of an inhibitor on USP11 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).

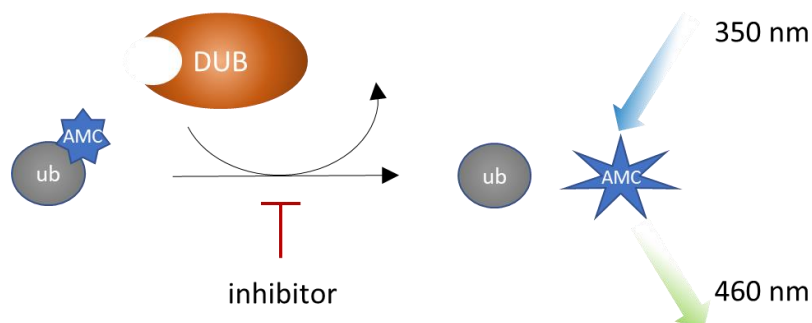


Figure 1: Illustration of the assay principle.

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 DUB activity.

Background

USP11 (ubiquitin specific protease 11), or ubiquitin carboxyl-terminal hydrolase 11, belongs to the USP family of proteins. USP11 is a DUB (deubiquitinating enzymes) and is involved in removing K-48 and K-63 ubiquitin chains from several substrates. It participates in DNA repair and inflammation, can regulate immune cell differentiation via the NF- κ B and TNF (tumor necrosis factor) pathways. It has been linked to the development and progression of several cancer types, such as breast, hepatocellular and ovarian cancer. The development of USP11 specific inhibitors will bring therapeutic benefits in cancer therapy.

Applications

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

Supplied Materials

| Catalog # | Name | Amount | Storage |
|-----------|---------------------------|----------|-----------|
| 102017 | USP11, His-Tag, FLAG-Tag* | 6 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 \(bpsbioscience.com\)](https://www.bpsbioscience.com/protein-faqs).
- 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.

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 the unused 1x Assay Buffer at the end of the day.
4. Thaw **USP11** on ice. Briefly spin the tube to recover its full content.
5. Dilute **USP11** to 2.4 ng/μl in 1x Assay Buffer (you need 25 μl/well).
6. Add 25 μl of **diluted USP11** to all wells, except “Negative Control”.
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 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.
11. Preincubate the Test inhibitor with the diluted USP11 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 μ l of **diluted Ub-AMC Substrate** to all wells.



Protect your samples from direct exposure to light.

14. Incubate at RT for 30 minutes.

| Component | Negative Control | Positive Control | Test Inhibitor |
|---------------------------------|-----------------------------|-----------------------------|-----------------------------|
| 1x Assay Buffer | 25 μ l | - | - |
| Test Inhibitor | - | - | 5 μ l |
| Diluent Solution | 5 μ l | 5 μ l | - |
| Diluted USP11 (2.4 ng/ μ l) | - | 25 μ l | 25 μ l |
| 30 minutes at Room Temperature | | | |
| Diluted Ub-AMC Substrate | 20 μ l | 20 μ l | 20 μ l |
| Total | 50 μl | 50 μl | 50 μl |

15. Read the fluorescence intensity of the samples ($\lambda_{\text{excitation}}$ = 350-380 nm; $\lambda_{\text{emission}}$ = 440-460 nm) in a fluorescence reader.

Example Results:

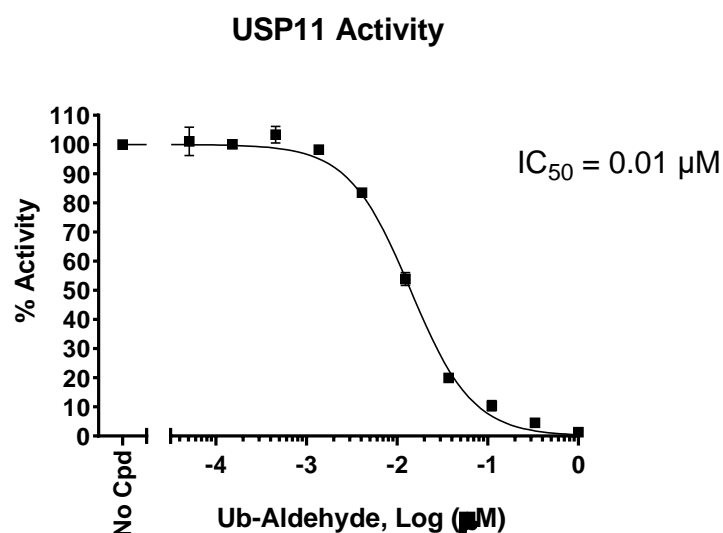


Figure 2. USP11 activity is inhibited by Ub-Aldehyde.

USP11 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 Y., *et al.*, 2022 *Cell Death Discovery* 8:292.

Related Products

| <i>Products</i> | <i>Catalog #</i> | <i>Size</i> |
|--------------------------------------------|------------------|--------------|
| 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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