Version 2b, Last updated 28 July 2023

ab241002 Deubiquitinase Assay Kit)

For the measurement of DUB activity in various tissues and cell extracts, and characterization of activity of purified DUB enzymes.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Table of Contents

1. Overview

Deubiquitinase Assay Kit (ab241002) provides a straight-forward and general measure of deubiquitinase activity by utilizing a fluorescent deubiquitinase substrate to detect activity as low as 0.25 µU with purified enzyme.

Fl-Substrate ⟶ Cleaved Substrate + Fluorescence

2. Protocol Summary

Prepare tissue or cell samples and positive control.

Prepare standard curve.

Prepare substrate mix and add to standards, positive control and sample wells.

Measure fluorescence (Ex/Em = 350/440 nm) immediately in kinetic mode for at least 30 min at 25°C.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
DUB Assay Buffer	25 mL	-20°C
DTT II/1 M DTT	100 µL	-20°C
DUB Substrate/DUB Substrate (in DMSO)	25 µL	-20°C
Active DUB Enzyme/DUB Positive Control	1 vial	-20°C
AMC Standard/AMC Standard (1 mM)	100 µL	-20°C
96-Well Half Area White Plate/White 96-well Half-Area Plate	1 unit	-20°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Multi-well spectrophotometer

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 DUB Assay Buffer:

Ready to use as supplied. Bring to room temperature before use. Store at -20°C.

6.2 DTT II/1 M DTT:

Ready to use as supplied. Bring to room temperature before use. Store at -20°C

6.3 DUB Substrate/DUB Substrate (in DMSO):

Ready to use. Warm DUB Substrate to room temperature before use. Light sensitive. Do not expose components to light for extended periods of time. Store at -20°C. Use within six months.

6.4 Active DUB Enzyme/DUB Positive Control:

Reconstitute with 22 µL of DUB Assay Buffer with DTT II/DTT to prepare the stock solution. Aliquot and store at -80°C. Avoid repeated freeze/thaw. Use within two months.

6.5 AMC Standard/AMC Standard (1 mM):

Ready to use. Warm AMC Standard to room temperature before use. Light sensitive. Do not expose components to light for extended periods of time. Store at -20°C. Use within six months.

6.6 96-Well Half Area White Plate/White 96-well Half-Area Plate:

Ready to use as supplied.

7. Standard Preparation

– Always prepare a fresh set of standards for every use.

7.1 To prepare AMC Standard, dilute 10 µL of AMC Standard/1 mM AMC Standard into 990 µl DUB Assay Buffer with DTT II/DTT to obtain a 10 µM stock concentration.

7.2 Add 0, 2, 4, 6, 8, and 10 µL of diluted standard into a series of wells in a 96-well plate and adjust the final volume to 50 µL/well with the DUB Assay Buffer with DTT II/DTT to generate 0, 20, 40, 60, 80, and 100 pmol/well AMC Standard. Mix well.

Standard #	AMC 10 µM Standard (µL)	DUB Assay Buffer (µL)	AMC Standard pmol/well
1	0	50	0
2	2	48	20
3	4	46	40
4	6	44	60
5	8	42	80
6	10	40	100

8. Sample Preparation

8.1 Add 1 µL of the supplied 1 M DTT II/DTT per ml DUB Assay Buffer for a 1 mM final DTT II/DTT concentration. The Assay Buffer is now ready to use. Make as much as necessary for number of experiments being run.

8.2 For tissue samples, add 50 µL ice-cold DUB Assay Buffer with 1 mM DTT II/DTT per mg of sample (wet weight). Homogenize on ice using a dounce homogenizer.

8.3 To prepare cell lysate, resuspend cells in ice-cold Assay Buffer with DTT II/DTT (10^5 cells per 50 µL) and homogenize using a dounce homogenizer.

8.4 Centrifuge lysate (tissue or cell) at 10,000 X *g* for 5 min. at 4°C.

8.5 Collect the supernatant.

8.6 Add 5-10 µL into a well of the provided half- area 96-well plate.

8.7 For the positive control reaction, use 2 µL of the reconstituted Active DUB Enzyme/Positive Control.

8.8 Adjust the volume of each reaction to 40 µL with DUB Assay Buffer (with DTT II/DTT).

Δ Note:

- For unknown samples, we recommend doing a pilot experiment and testing several doses to ensure the readings are within the Standard Curve range.
- For samples having high background, prepare parallel sample background controls (by omitting the substrate mix and instead adding 10 µL DUB Assay Buffer with DTT II/DTT).

9. Assay Procedure

9.1 Prepare Substrate Mix by diluting the stock solution to a working concentration. Add 25 µL substrate to 1075 µL DUB Assay Buffer with DTT II/DTT for the working stock (dilution factor = 1:44).

Δ Note: If lower numbers of wells (for example, 20) are needed, 5 µL of DUB substrate can be diluted into 215 µL DUB Assay Buffer with DTT II/DTT.

Δ Note: Use diluted substrate within 4 hours.

9.2 To initiate reaction, add 10 µL substrate mix to each well.

9.3 Measure fluorescence at 25°C in kinetic mode for at least 30 min.

9.4 Incubation time depends on the DUB activity in the samples. We recommend measuring the reaction progress in kinetic mode and choosing two time points (T1 and T2) in the linear range to calculate the DUB activity of the samples. The AMC Standard Curve can be read in endpoint mode (i.e. at the end of incubation time).

10. Data Analysis

10.1 Subtract 0 Standard reading from all readings.
10.2 Plot the AMC Standard Curve.
10.3 If sample background control reading is significant, subtract the background control reading from its paired sample reading.
10.4 Calculate the DUB activity of the test sample: ΔRFU = RFU2 – RFU1.
10.5 Apply the ΔRFU to the AMC Standard Curve to get B pmol of AMC generated during the reaction time (Δt = t2 - t1).

Sample DUB Activity = **B/(ΔT x V) x D** = pmol/min/mL = µU/mL

Where:

B is AMC amount in the sample well from Standard Curve (pmol).
ΔT is reaction time (min).
V is sample volume added into the reaction well (mL)
D is sample dilution factor

Unit Definition: One unit of DUB that can cleave 1 µmol of substrate/min under the assay conditions at 25°C.

11. Typical Data

Typical data provided **for demonstration purposes only**.

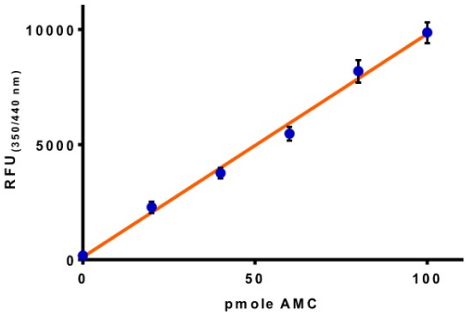

Figure 1. AMC Standard Curve.

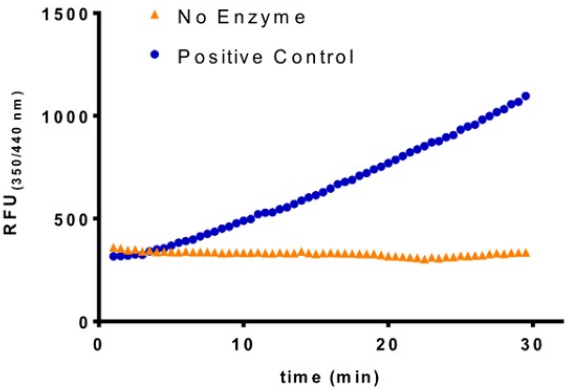

Figure 2. Time course using Active DUB Enzyme/positive control DUB as described.

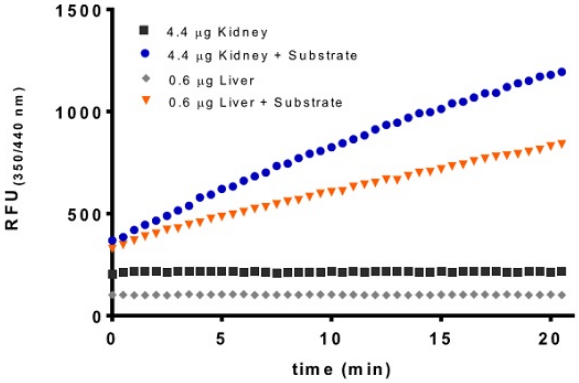

Figure 3. Example of determination of DUB activity in tissue lysates. Rat tissue samples (10 mg each) were resuspended in DUB Assay Buffer with DTT II/DTT (100µL), homogenized, and clarified by centrifugation. The DUB activities for rat kidney and liver lysates, in mU/mg, were determined to be 0.76 and 3.31, respectively.

13. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)