

Version 2f, Last updated 23 June 2025

# **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.

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## 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  $\mu$ U with purified enzyme.

Fl-Substrate  $\longrightarrow$  Cleaved Substrate + Fluorescence

## 2. Protocol Summary

Prepare tissue or cell samples and positive control.



Prepare standard curve.



Prepare substrate mix and add to 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](http://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	100 µL	-20°C
DUB Substrate	25 µL	-20°C
Active DUB Enzyme	1 vial	-20°C
AMC Standard	100 µL	-20°C
96-Well Half Area White 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:

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

### 6.3 DUB Substrate:

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:

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

### 6.5 AMC Standard:

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:

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  $\mu\text{L}$  of AMC Standard/1 mM AMC Standard into 990  $\mu\text{L}$  DUB Assay Buffer with DTT II to obtain a 10  $\mu\text{M}$  stock concentration.
- 7.2** Add 0, 2, 4, 6, 8, and 10  $\mu\text{L}$  of diluted standard into a series of wells in a 96-well plate and adjust the final volume to 50  $\mu\text{L}$ /well with the DUB Assay Buffer with DTT II to generate 0, 20, 40, 60, 80, and 100 pmol/well AMC Standard. Mix well.

<b>Standard #</b>	<b>AMC 10 <math>\mu\text{M}</math> Standard (<math>\mu\text{L}</math>)</b>	<b>DUB Assay Buffer (<math>\mu\text{L}</math>)</b>	<b>AMC Standard pmol/well</b>
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  $\mu\text{L}$  of the supplied 1 M DTT II per ml DUB Assay Buffer for a 1 mM final DTT II 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  $\mu\text{L}$  ice-cold DUB Assay Buffer with 1 mM DTT II 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 ( $10^5$  cells per 50  $\mu\text{L}$ ) 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  $\mu\text{L}$  into a well of the provided half- area 96-well plate.
- 8.7 For the positive control reaction, use 2  $\mu\text{L}$  of the reconstituted Active DUB Enzyme.
- 8.8 Adjust the volume of each reaction to 40  $\mu\text{L}$  with DUB Assay Buffer (with DTT II).

### **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  $\mu\text{L}$  DUB Assay Buffer with DTT II).

## 9. Assay Procedure

- 9.1** Prepare Substrate Mix by diluting the stock solution to a working concentration. Add 1075  $\mu\text{L}$  DUB Assay Buffer with DTT II to the 25  $\mu\text{L}$  substrate for the working stock (dilution factor = 1:44).
- Δ Note:** If lower numbers of wells (for example, 20) are needed, 5  $\mu\text{L}$  of DUB substrate can be diluted into 215  $\mu\text{L}$  DUB Assay Buffer with DTT II.
- Δ Note:** Use diluted substrate within 4 hours.
- 9.2** To initiate reaction, add 10  $\mu\text{L}$  substrate mix to each sample and positive control 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:  $\Delta\text{RFU} = \text{RFU}_2 - \text{RFU}_1$ .
- 10.5 Apply the  $\Delta\text{RFU}$  to the AMC Standard Curve to get B pmol of AMC generated during the reaction time ( $\Delta t = t_2 - t_1$ ).

$$\text{Sample DUB Activity} = \mathbf{B}/(\mathbf{\Delta T} \times \mathbf{V}) \times \mathbf{D} = \text{pmol}/\text{min}/\text{mL} = \mu\text{U}/\text{mL}$$

### Where:

**B** is AMC amount in the sample well from Standard Curve (pmol).

**$\Delta\text{T}$**  is reaction time (min).

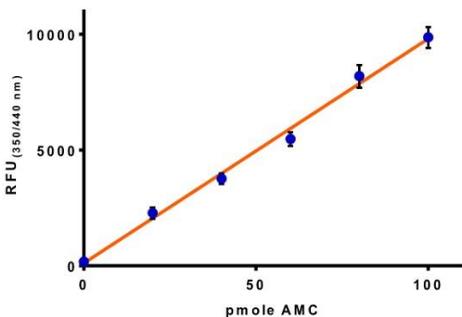
**V** is sample volume added into the reaction well (mL)

**D** is sample dilution factor

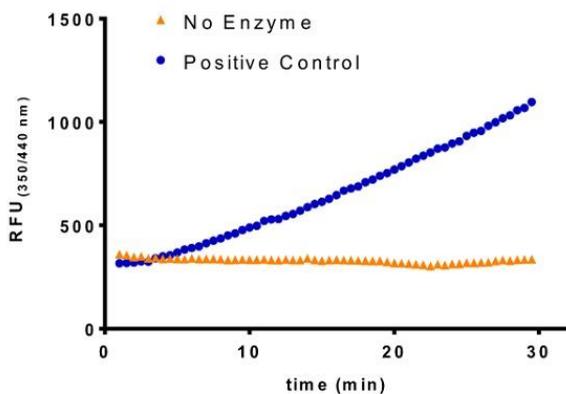
**Unit Definition:** One unit of DUB that can cleave 1  $\mu\text{mol}$  of substrate/min under the assay conditions at 25°C.

## 11. Typical Data

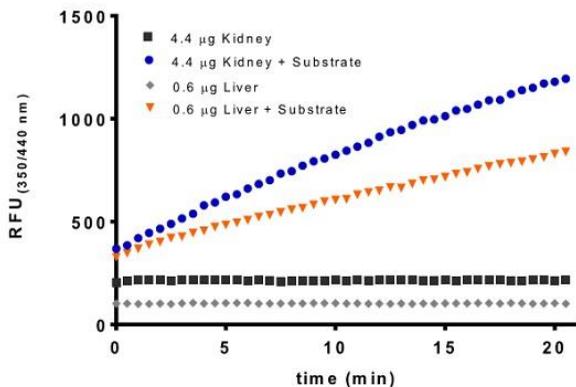
Typical data provided for demonstration purposes only.



**Figure 1.** AMC Standard Curve.



**Figure 2.** Time course using Active DUB Enzyme 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 (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



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