

ab284549 – HDAC6 Activity Assay Kit (Fluorometric)

For the measurement of HDAC6 activity.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab284549>

Storage and Stability

All components in this kit are shipped on blue ice and are suitable for storage at -80°C, unless reconstituted. Upon receipt, immediately store kit at -80°C in the dark. Individual components may be stored at alternative temperatures as shown in the table below. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted. Upon opening, use kit within 6 months.

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer 39	25 mL	-80°C or -20°C
HDAC6 Lysis Buffer	25 mL	-80°C or -20°C
HDAC6 Positive Control	10 µl	-80°C
HDAC Substrate III	200 µl	-80°C or -20°C
Developer Mix M	1 mL	-80°C
HDAC6 Inhibitor (Tubacin)	20 µl	-80°C or -20°C
AFC Standard	100 µl	-80°C or -20°C

PLEASE NOTE: Developer Mix M was previously labelled as Developer II and Developer, and HDAC6 Inhibitor (Tubacin) as AFC Standard (1 mM) and HDAC6 Inhibitor (Tubacin, 1 mM). Assay Buffer 39 was previously labelled as Assay Buffer XXXIX and HDAC6 Assay Buffer. The composition has not changed.

Materials Required, Not Supplied

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

- Multi-well spectrophotometer
- 96-well white plate
- DMSO

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Assay Buffer 39 and Lysis Buffer: Warm to room temperature (RT) before use.

HDAC Substrate III and HDAC6 Inhibitor (Tubacin): Store at -20°C. Dilute necessary amount of HDAC6 Inhibitor (Tubacin) 10X with DMSO before use.

Developer Mix M: Thaw on ice before use.

HDAC6 Positive Control: Thaw on ice before use. Divide into aliquots and store at -80°C. Thaw on ice before use. Avoid repeated freeze/thaw cycles. Unused enzyme must be stored at -80°C immediately.

Assay Protocol

Sample Preparation:

1. Homogenize fresh or frozen tissue (~5-10 mg) or cells (1-2 x 10⁶) with 100 µl HDAC6 Lysis Buffer on ice and incubate on ice for 5 min. Centrifuge the homogenate at 16,000 X g, 4°C for 10 min.
2. Transfer the clarified supernatant to a fresh prechilled tube and keep on ice. Measure the amount of protein in the lysate or purified enzyme using any preferred method. We recommend using BCA Protein Assay Kit.
3. Add 1-10 µl of lysate or purified enzyme into desired well(s) in a white 96- well plate. If necessary, dilute the lysate with Assay Buffer 39.
4. As an Inhibitor Control, run a parallel sample containing same amount of lysate in another well containing 2 µl of 10X diluted HDAC6 Inhibitor (Tubacin) and incubate at 37°C for 10 min.
5. For Positive Control, dilute 2 µl of Human HDAC6 with 48 µl of Assay Buffer 39 and use 25 µl/well.
6. Adjust the volume of Samples and Positive Control to 50 µl/well with Assay Buffer 39.

Δ Notes:

- a. We recommend using the Samples for activity analysis immediately. Otherwise, store the Sample(s) at -80°C for 3-4 days.
- b. For Unknown Samples, we suggest testing several concentrations to ensure that the readings are within the Standard Curve range
- c. Cells can also be grown in the presence and absence of Tubacin (2 µM), lysed using HDAC6 Lysis Buffer and tested directly in the assay.

Standard Curve Generation:

1. Dilute AFC Standard to 20 µM by adding 10 µl of 1 mM AFC Standard to 0.5 ml of Assay Buffer 39.
2. Add 0, 5, 10, 15, 20, and 25 µl of diluted 20 µM AFC Standard into a series of wells in a 96-well white plate and adjust the final volume to 100 µl/well with Assay Buffer 39 to generate 0, 100, 200, 300, 400, and 500 pmol/well of AFC Standard respectively. Mix well.

Substrate Mix:

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl substrate mix containing:

Item	Substrate Mix
Assay Buffer 39	48 µl
HDAC Substrate III	2 µl

2. Mix well and add 50 µl of the substrate mix to wells containing the Sample, and Positive Control.

Δ Note: Do not add Substrate Mix to the sample Background Control and Standard wells.

Incubation and Measurement

1. Cover the plate and incubate at 37°C for 30 min.
2. Add 10 µl of Developer Mix M to each well to stop reaction.
3. Mix well, cover the plate and incubate at 37°C for 10 min to generate fluorescence. The fluorescence signal is stable for at least 30 min after the addition of Developer Mix M.
4. Measure the fluorescence at Ex/Em 380/490 nm in an end point mode at 37°C.

Calculation

- Obtain Δ RFU for all test compounds by subtracting fluorescence of the 0 Standard Controls from those containing all standards. Plot the Δ RFU against pmol of AFC. The plot should be linear; determine the slope A (Δ RFU/pmol) of the curve.
- Calculate Δ RFU (B) for each Sample by subtracting fluorescence of corresponding Inhibitor Control. Using this value, calculate Sample HDAC6 activity using following equation.

$$\text{Sample HDAC6 Activity } \left(X, \frac{U}{ml} \right) = \frac{B \times 1000}{A \times T \times V} \times \text{Dilution Factor}$$

$$\text{Sample HDAC6 Activity } \left(\frac{U}{mg} \right) = \frac{X}{P}$$

Where: **B** = Sample HDAC6 Activity as calculated (Δ RFU)
A = Slope of the AFC standard curve (Δ RFU/pmol)
T = 30 = Assay Time (min)
V = μ l of Sample used in the assay
P = Protein concentration in the lysate (mg/ml)

Unit Definition: 1 U is the amount of HDAC6 required to deacetylate 1 pmol of HDAC Substrate III per min under the assay conditions.

Technical Support

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