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HDAC6 Activity Assay Kit (Fluorometric)

rev 07/20

(Catalog # K466-100; 100 assays, Store kit at -80°C)

I. Introduction:

Histone Deacetylase 6 (HDAC6, EC 3.5.1.98), is a class IIb HDAC enzyme that deacetylates a ε-N-acetyl lysine of histone and non-histone protein substrates. It is a unique member of HDAC family as it contains two deacetylase domains that are proposed to function independent of each other. HDAC6 can shuttle between the nucleus and cytoplasm, suggesting potential extra-nuclear functions by regulating the acetylation status of non-histone substrates. HDAC6 also affects transcription and translation by regulating the heat-shock protein 90 (Hsp90) and stress granules. Elevated HDAC6 activity is associated with cell motility and increases α-tubulin deacetylation, thus influencing cancer cell metastasis. In addition, mutations in HDAC6 gene have been associated with Alzheimer's disease. HDAC6-selective inhibitors are considered as promising targets for autoimmune, oncology and inflammatory diseases. **BioVision's HDAC6 Activity Assay Kit** utilizes deacetylase activity of HDAC6 towards a synthetic acetylated-peptide substrate resulting in the release of an AFC fluorophore, which can be easily quantified using a conventional microplate reader. This simple and high-throughput adaptable assay kit can be used to detect HDAC6 activity (as low as 3 U) in complex biological samples.

HDAC6 Substrate-AFC $\xrightarrow[\text{2. Developer}]{\text{1. HDAC6}}$ Cleaved Substrate + AFC Fluorescence (Ex/Em = 380/490 nm)

II. Application:

- Detection of deacetylase activity of HDAC6 in biological samples such as tissue, cell lysates, etc.

III. Sample Type:

- Recombinant protein, tissue, cell lysates, etc.

IV. Kit Contents:

Components	K466-100	Cap Code	Part Number
HDAC6 Assay Buffer	25 ml	WM	K466-100-1
HDAC6 Lysis Buffer	25 ml	NM	K466-100-2
Human HDAC6 Positive Control	1 vial	Green	K466-100-3
HDAC6 Substrate	0.2 ml	Red	K466-100-4
Developer	1 ml	Orange	K466-100-5
HDAC6 Inhibitor (Tubacin, 1 mM)	20 µl	Blue	K466-100-6
AFC Standard (1 mM)	100 µl	Yellow	K466-100-7

V. User Supplied Reagents and Equipment:

- 96-well white well plate
- Multi-well spectrofluorometer
- DMSO

VI. Storage Conditions and Reagent Preparation:

Store the entire kit at -80°C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

- **HDAC6 Assay and Lysis Buffer**: Bring to room temperature before use. Store at 4°C or -20°C.
- **HDAC6 Substrate and Inhibitor**: Store at -20°C. Dilute necessary amount of Inhibitor 10X with DMSO before use.
- **Developer**: Store at -20°C. Thaw on ice before use.
- **Human HDAC6 Positive Control**: Provided as liquid. Divide into aliquots and store at -80°C. Thaw on ice before use. Avoid repeated freeze/thaw cycles. Unused enzyme must be store at -80°C immediately.

VII. HDAC6 Activity Assay Protocol:

1. **Sample Preparation:** Homogenize fresh or frozen tissue (~5-10 mg) or cells (1-2 x 10^6) 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. Transfer the clarified supernatant to a fresh pre-chilled tube and keep on ice. Measure the amount of protein in the lysate or purified enzyme using BCA Protein Assay Kit - Reducing Agent Compatible (BioVision Cat# K818-1000 or equivalent). 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 HDAC6 Assay buffer. As an **Inhibitor Control**, run a parallel sample containing same amount of lysate in another well containing 2 µl of 10X diluted HDAC6 inhibitor (in DMSO) and incubate at 37°C for 10 min. For **Positive Control**, dilute 2 µl of Human HDAC6 with 0.5 ml of HDAC6 Assay Buffer and use 25-50 µl/well. Adjust the volume of Samples and Positive Control to 50 µl/well with HDAC6 Assay Buffer.

   Notes:

   a. We recommend using the tissue/cell homogenate immediately to measure the HDAC6 activity. If desired, snap freeze the lysate and store at -80°C.

   b. For Unknown Samples, we suggest doing pilot experiment and testing 3-5 different amounts of samples to ensure 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 (Haggarty *et. al.*, *PNAS*, **2003**, 100 (8), 4389-4394).

d. **Optional**: For samples having background, prepare parallel sample well(s) as sample background control. Use same amount of tissue/cell homogenate or purified enzyme as in the sample well. Adjust the final volume to 100 µl with HDAC6 Assay Buffer.

2. **Standard Curve Preparation:** Dilute AFC Standard to 20 µM by adding 10 µl of 1 mM AFC Standard to 0.5 ml of HDAC6 Assay Buffer. 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 HDAC6 Assay Buffer to generate 0, 100, 200, 300, 400, and 500 pmol/well of AFC Standard respectively. Mix well.

3. **HDAC6 Substrate Mix Preparation**: Prepare 50 µl of HDAC6 Substrate Mix per well as given below:

48 µl HDAC6 Assay Buffer
2 µl HDAC6 Substrate

Dissolve the Substrate Mix by vigorous vortexing. Add 50 µl of Substrate Mix solution into each Sample, and Positive Control well.

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

4. **Incubation and Measurement**: Mix well, cover the plate and incubate at 37°C for 30 min. To stop the reaction, add 10 µl of Developer to each well. 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. Measure the fluorescence at Ex/Em 380/490 nm in an end point mode at 37°C.

5. **Calculations:**

a. **AFC Standard Curve**: Obtain change in the RFU (ΔRFU) by subtracting fluorescence of the 0 Standard Controls from those containing all standards. Plot the ΔRFU against pmol of AFC. The plot should be linear; determine the slope **A** (ΔRFU/pmol) of the curve.

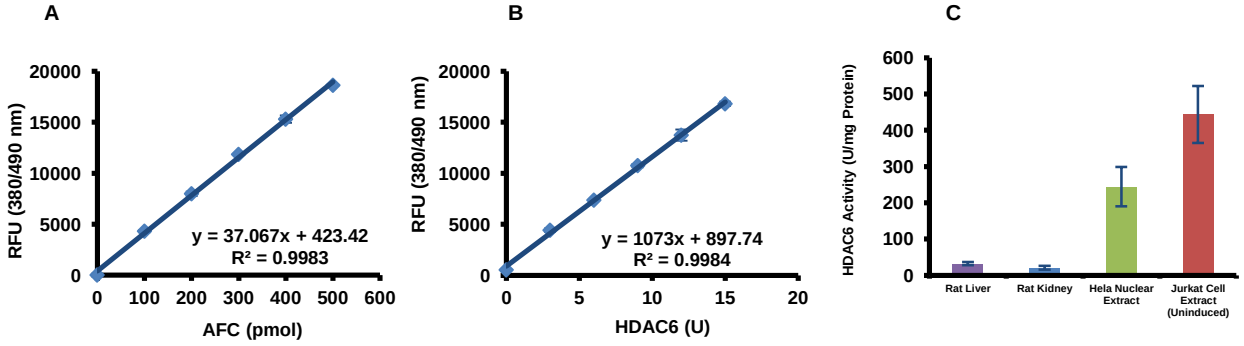
b. **Samples**: Calculate ΔRFU (**B**) for each Sample by subtracting fluorescence of corresponding Inhibitor Control. Using this value, calculate Sample HDAC6 activity using following equation.

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

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

Where, **B** = Sample HDAC6 Activity as calculated (ΔRFU)
A = Slope of the AFC standard curve (ΔRFU/pmol)
T = **30** = Assay Time (min)
V = µl 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 HDAC6 Substrate per min under the assay conditions.



Figures: AFC Standard Curve (A), HDAC6 activity with different amounts of Positive Control (B), and in rat liver, kidney lysates along with Hela Nuclear Extract (Cat. No. 1641), uninduced (Cat. No. 1106) and Camptothecin-induced Jurkat Cell Extracts (Cat. No. 1107) (C) are shown in the figure (n = 3). The assays were performed according to the kit protocol.

VIII. RELATED PRODUCTS:

HDAC Activity Colorimetric Assay Kit (K331)
HDAC Inhibitor Drug Screening Kit (Fluorometric) (K340)
HDAC2 Immunoprecipitation (IP) & Activity Assay Kit (K341)
HDAC3 Immunoprecipitation (IP) & Activity Assay Kit (K344)
HDAC8 Activity Fluorometric Assay Kit (K348)
HeLa Nuclear Extract (1641)
Jurkat Cell Extract (Induced) (1107)
HDAC Activity Fluorometric Assay Kit (K330)
HDAC1 Immunoprecipitation (IP) & Activity Assay Kit (K342)
HDAC3 Activity Fluorometric Assay Kit (K343)
HDAC3 Inhibitor Screening Kit (Fluorometric) (K363)
HDAC8 Inhibitor Screening Kit (Fluorometric) (K368)
Jurkat Cell Extract (Uninduced) (1106)

FOR RESEARCH USE ONLY! Not to be used on humans.