

# Fluorometric HDAC Activity Assay Kit

(Catalog #K330-100; 100 assays; Store kit at  $-20^{\circ}\text{C}$ )

## I. Introduction:

Inhibition of histone deacetylase (HDAC) has been implicated to modulate transcription and induce apoptosis or differentiation in cancer cells. However, screening compounds for HDAC inhibition has been difficult due to the lack of convenient tools for analyzing HDAC activity. The Fluorometric HDAC Activity Assay Kit provides a fast and fluorescence-based method that eliminates radioactivity, extractions, or chromatography, as used in traditional assays. The new procedure requires only two easy steps, both performed on the same microtiter plate. First, the HDAC substrate, which comprises an acetylated lysine side chain, is incubated with a sample containing HDAC activity (e.g., HeLa nuclear extract). Deacetylation of the substrate sensitizes the substrate, so that further treatment with the Lysine Developer produces a fluorophore. The fluorophore can be easily analyzed using a fluorescence plate reader or a fluorometer. The assay is well suited for high throughput screening applications. HDAC inhibitors and antibodies are also available separately.

## II. Kit Contents:

Component	K330-100	Color Code
	100 assays	Cap Color
HDAC Substrate [Boc-Lys(Ac)-AMC, 4 mM]	500 $\mu\text{l}$	Amber
10X HDAC Assay Buffer	1.0 ml	Green
Lysine Developer	1.0 ml	Orange
HDAC Inhibitor (Trichostatin A, 1 mM)	10 $\mu\text{l}$	Blue
HeLa Nuclear Extract (5 mg/ml)	10 $\mu\text{l}$	Red
Deacetylated Standard [Boc-Lys-AMC, 4 mM]	20 $\mu\text{l}$	Yellow

## III. Histone Deacetylase Assay Protocol:

### A. General Consideration:

- Read the entire protocol before beginning the procedure.
- The HeLa extract should be refreeze immediately at  $-70^{\circ}\text{C}$  after each use to avoid loss of activity.
- After opening the kit, the Lysine Developer is recommended to be aliquoted and refreeze at  $-20^{\circ}\text{C}$  for future use.
- If positive and negative controls are designed, the kit provides sufficient reagents for 5 positive control assays with the HeLa Nuclear Extract and 5 Negative Control assays with the HDAC Inhibitor, Trichostatin A.

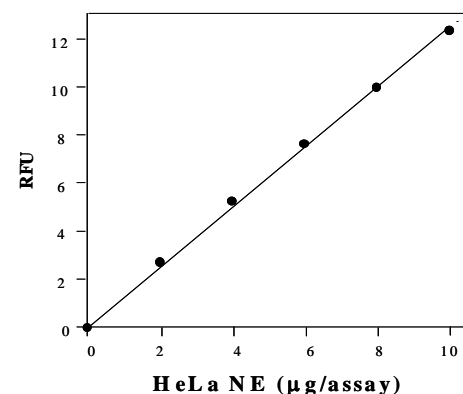
### B. Assay Protocol:

1. Dilute test samples (10-50  $\mu\text{g}$  of nuclear extract or cell lysate) to 85  $\mu\text{l}$  (final volume) of ddH<sub>2</sub>O in each well (For background reading, add 85  $\mu\text{l}$  ddH<sub>2</sub>O only). For positive control, dilute 2  $\mu\text{l}$  of HeLa nuclear extract with 83  $\mu\text{l}$  ddH<sub>2</sub>O. For negative control, dilute the sample into 83  $\mu\text{l}$  of ddH<sub>2</sub>O and then add 2  $\mu\text{l}$  of Trichostatin, or use a known sample containing no HDAC activity.

2. Add 10  $\mu\text{l}$  of the 10X HDAC Assay Buffer to each well.
3. Add 5  $\mu\text{l}$  of the HDAC Fluorometric Substrate to each well. Mix thoroughly.
4. Incubate plates at  $37^{\circ}\text{C}$  for 30 minutes (or longer if desired).
5. Stop the reaction by adding 10  $\mu\text{l}$  of Lysine Developer and mix well. Incubate the plate at  $37^{\circ}\text{C}$  for 30 min.
6. Read sample in a fluorescence plate reader with Ex. = 350-380 nm and Em. = 440-460 nm. Signal should be stable for several hours at room temperature. Histone Deacetylase activity can be expressed as the Relative Fluorescence Units per  $\mu\text{g}$  protein sample.

### C. Standard Curve (optional):

1. If desired, a standard curve can be prepared using the known amount of the Deacetylated Standard included in the kit. The exact concentration range of the Deacetylase Standard will vary depending on the fluorometer model, the gate setting, and the exact wavelength used. We recommend starting with a dilution range of 1-20  $\mu\text{M}$  in Assay Buffer.
2. Add 90  $\mu\text{l}$  each of the dilutions and also 10  $\mu\text{l}$  of the 10X Assay Buffer into a set of wells on the microtiter plate. Use 90  $\mu\text{l}$  of H<sub>2</sub>O and 10  $\mu\text{l}$  of 10X Assay Buffer as zero.
3. Add 10  $\mu\text{l}$  of Lysine Developer to each well and incubate at  $37^{\circ}\text{C}$  for 30 min (Note: Incubation time should be kept the same for both standard and test samples.)
4. Read samples in a fluorescence plate reader or a fluorometer with Ex. = 350-380 nm and Em. = 440-460 nm.
5. Plot fluorescence signal (y-axis) versus concentration of the Deacetylated Standard (x-axis). Determine the slope as AFU/ $\mu\text{M}$ .
6. Based on the slope, you can determine the absolute amount of deacetylated lysine generated in your sample.



**Analyses of HDAC Activity in HeLa Nuclear Extract.** HeLa nuclear extract (NE) in various amounts were incubated with 5  $\mu\text{l}$  HDAC fluorometric substrate. After 30 min, reactions were stopped with 10  $\mu\text{l}$  Lysine Developer. Samples were then read in a fluorescence plate reader with Ex./Em. = 360/460 nm.