Introduction and Background

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

Material and Method

A. List of component

1. HDAC Substrate [Boc-Lys(Ac)- pNA, 10 mM]: 500 µl
2. 10X HDAC Assay Buffer: 1 ml.
3. Lysine Developer: 1 ml.
4. HDAC Inhibitor (Trichostatin A, 1 mM) : 10 µl
5. HeLa Nuclear Extract (5 mg/ml): 10 µl.
6. Deacetylated Standard [(Boc-Lys-pNA, 10 mM]: 20 µl.

B. General Consideration

- Read the entire protocol before beginning the procedure.
- The HeLa nuclear extract should be refreeze immediately at -70℃ after each use to avoid loss of activity.
- The kit provides sufficient reagents for 100 positive control assays with the HeLa Nuclear Extract and 5 Negative Control assays with the HDAC Inhibitor, Trichostatin A.

C. Assay Protocol

1. Dilute test samples into 85 µl (final volume) with ddH_2O in each well. For positive reading, add 85 µl ddH_2O only. For negative reading, add 83 µl of ddH_2O and 2 µl of Trichostatin.
2. Add 10 µl of the 10X HDAC Assay Buffer and 2 µl of HeLa Nuclear Extract to each well. Mix thoroughly.
3. Add 5 µl of the HDAC Fluorometric Substrate to each well. Mix thoroughly. Incubate plates at 37℃ for 30 minutes (or longer if desired).
4. Stop the reaction by adding 10 µl of Lysine Developer and mix well. Incubate the plate at 37℃ for 30 min.

5. 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. Inhibitor activity can be expressed as inhibition of Relative Fluorescence Units.

D. 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 µM in Assay Buffer.
2. Add 90 µl each of the dilutions and also 10 µl of the 10X Assay Buffer (as zero) into a set of wells on the microtiter plate.
3. Add 10 µl of Lysine Developer to each well and incubate at 37℃ 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/µM.
6. Based on the slope, you can determine the absolute amount of deacetylated lysine generated in your sample.