KAMIYA BIOMEDICAL COMPANY

HDAC Inhibitor Drug Screening Kit

For the effective screening of HDAC inhibitors using a fluorometric method.

Cat. No. KT-145

For research use only, not for use in diagnostic procedures.

PRODUCT INFORMATION

HDAC Inhibitor Drug Screening Kit

Cat. No. KT-145
(100 tests)

PRODUCT

The ***K-ASSAY*®** HDAC Inhibitor Drug Screening Kit is a convenient fluorometric assay to screen HDAC inhibitor samples.

PRINCIPLE

Inhibition of histone deacetylase (HDAC) has been implicated to modulate transcription and to induce apoptosis or differentiation in cancer cells. However, screening of compounds for HDAC inhibition has been difficult due to the lack of convenient tools for analyzing HDAC activity. The new HDAC Inhibitor Drug Screening 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 fluorometric substrate, which comprises an acetylated lysine side chain, is incubated with a sample containing HDAC activity (e.g., HeLa nuclear extract or others). Deacetylation of the substrate sensitizes the substrate, so that, in the second step, 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.

COMPONENTS

• HDAC Substrate [Boc-Lys(Ac)-AMC, 4 mM]	500 µl	Amber cap
• 10X HDAC Assay Buffer	1.0 ml	Green cap
• Lysine Developer	1.0 ml	Orange cap
• HDAC Inhibitor (Trichostatin A, 1 mM)	10 µl	Blue cap
• HeLa Nuclear Extract (5 mg/ml)	200 µl	Red cap
• Deacetylated Standard [Boc-Lys-AMC, 4 mM]	20 µl	Yellow cap

PROTOCOLS

A. General Consideration

- Read the entire protocol before beginning the procedure.
- The HeLa extract should be refrozen immediately at –70°C after each use to avoid the 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.

B. Assay Procedure

1. Dilute test samples (10-50 µg of nuclear extract or cell lysate) into 85 µl (final volume) of ddH_20 in

each well (For background reading, add 85 µl ddH_20 only). For positive control, dilute 2 µl of HeLa nuclear extract with 83 µl ddH_20. For negative control, dilute the sample into 83 µl of ddH_20 and then add 2 µl of Trichostatin, or use a known sample containing no HDAC activity.
2. Add 10 µl of the 10X HDAC Assay Buffer to each well.
3. Add 5 µl of the HDAC Fluorometric Substrate to each well. Mix thoroughly.
4. Incubate plates at 37°C for 30 minutes (or longer if desired).
5. Stop the reaction by adding 10 µl of Lysine Developer and mix well. Incubate the plate at 37°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 µg protein sample.

C. Standard Curve (optional)

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.

1. 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.
2. Add 10 µl of Lysine Developer to each well and incubate at 37°C for 30 min (Note: Incubation time should be kept the same for both standard and test samples.)
3. Read samples in a fluorescence plate reader or a fluorometer with Ex. = 350-380 nm and Em. = 440-460 nm.
4. Plot fluorescence signal (y-axis) versus concentration of the Deacetylated Standard (x-axis).
5. Determine the slope as AFU/µM.
6. Based on the slope, you can determine the absolute amount of deacetylated lysine generated in your sample.

STORAGE

Store Hela Nuclear Extract at -70°C and the rest of the kit at -20°C.

FOR RESEARCH USE ONLY

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