KAMIYA BIOMEDICAL COMPANY

HDAC Colorimetric Assay

For the fast and convenient measurement of HDAC activity.

Cat. No. KT-144

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

PRODUCT INFORMATION

HDAC Colorimetric Assay

Cat. No. KT-144
100 tests

PRODUCT

The ***K-ASSAY*** ® HDAC Colorimetric Assay is a fast and convenient colorimetric assay to measure HDAC activity in nuclear extract and other samples.

PRINCIPLE

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 new HDAC Colorimetric Assay Kit 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, substrates, and antibodies are also available separately.

COMPONENT

• HDAC Substrate [Boc-Lys(Ac)-pNA, 10 mM]	500 µl	(Amber cap)
• 10X HDAC Assay Buffer	1 ml	(Green cap)
• Lysine Developer	1 ml	(Orange cap)
• HDAC Inhibitor (Trichostatin A, 1 mM)	10 µl	(Blue cap)
• Hela Nuclear Extract (5 mg/ml)	50 µl	(Red cap)
•Deacetylated Standard (Boc-Lys-pNA, 10 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 –20 °C or -70°C after each use to avoid loss of activity.
- The Lysine Developer should refrozen immediately at –20 °C or -70°C after each use or aliquot for future use.

• If positive and negative controls are desired, 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 (50-200 µg of nuclear extract or cell lysate) to 85 µl (final volume) of ddH_20 in each well (For background reading, add 85 µl ddH_20 only). For positive control, dilute 10 µl of HeLa nuclear extract with 75 µl ddH_20. For negative control, dilute your 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 colorimetric substrate to each well. Mix thoroughly.
4. Incubate plates at 37°C for 1 hour (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 an ELISA plate reader at 400 or 405 nm. Signal is stable for several hours at room temperature. HDAC activity can be expressed as the relative O.D. value per µg 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 Deacetylated Standard will vary depending on the each individual plate reader and the exact wavelength used. We recommend starting with a dilution range of 10-100 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°C for 30 min (Note: Incubation time should be kept the same for both standard and test samples.)
4. Read samples in an ELISA plate reader at 400 or 405 nm.
5. Plot O.D. value (y-axis) versus concentration of the Deacetylated Standard (x-axis). Determine the slope as ΔO.D./µM.
6. Based on the slope, you can determine the absolute amount of deacetylated lysine generated in your sample.

STORAGE

Store at -20°C.

FOR RESEARCH USE ONLY

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