



Histone Deacetylase Colorimetric Assay Kit

Manufactured by BioVision.

ALX-850-294-KI01

~100 tests

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For laboratory use only. Not for human or diagnostic use.

I. Introduction:

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 Colorimetric HDAC Activity 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.

II. Kit Contents:

Component	ALX-850-294	Color Code
	100 tests	Cap Color
HDAC Substrate [Boc-Lys(Ac)-pNA, 10 mM]	500 μ l	Amber
10x HDAC Assay Buffer	1 ml	Green
Lysine Developer	1 ml	Orange
HDAC Inhibitor (Trichostatin A, 1 mM)	10 μ l	Blue
HeLa Nuclear Extract (5 mg/ml)	50 μ l	Red
Deacetylated Standard (Boc-Lys-pNA, 10 mM)	20 μ l	Yellow

III. HDAC Assay Protocol:

A. General Considerations:

- Read the entire protocol before beginning the procedure.
- Refreeze the HeLa nuclear extract immediately at -80°C after each use to avoid loss of activity.
- Refreeze the Lysine Developer immediately at -80°C after each use to avoid loss of activity or aliquot 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.
- We recommend using the 96-well plates with U-shape bottom. Flat bottom may give low value.

B. Assay Protocol:

1. Dilute test samples (50-200 μ g of nuclear extract or cell lysate) to 85 μ l (final volume) of ddH₂O in each well (For background reading, add 85 μ l ddH₂O only).

For positive control, dilute 10 μl of HeLa nuclear extract with 75 μl ddH₂O. For negative control, dilute your sample into 83 μl of ddH₂O 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 minutes.
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 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 into a set of wells on the microtiter plate. Use 90 μl of H₂O and 10 μl of 10x Assay Buffer as zero.
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 $\Delta\text{O.D.}/\mu\text{M}$.
6. Based on the slope, you can determine the absolute amount of deacetylated lysine generated in your sample.

V. Storage:

- Store kit at -80°C.

IV. References:

1. Strahl, B.D. & Allis, C.D. (2000) Nature 403:401.
2. Grunstein, M. (1997) Nature 389:249.
3. Cheung, W.L., et al. (2000) Curr Opin Cell Biol 12:326