# HDAC Activity Colorimetric Assay Kit

**(Catalog #K331-100; 100 assays; Store kit at –80°C)**

## 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 and antibodies are also available separately.

## II. Kit Contents:

| Component | K331-100<br>100 assays | Cap Color | Part Number |
|---|---|---|---|
| HDAC Substrate | 500 µl | Amber | K331-100-1 |
| 10X HDAC Assay Buffer | 1.0 ml | Green | K331-100-2 |
| Lysine Developer | 1.0 ml | Orange | K331-100-3 |
| HDAC Inhibitor (Trichostatin A, 1 mM) | 10 µl | Blue | K331-100-4 |
| HeLa Nuclear Extract (5 mg/ml) | 50 µl | Red | K331-100-5 |
| Deacetylated Standard (10 mM) | 20 µl | Yellow | K331-100-6 |

## III. General Consideration:

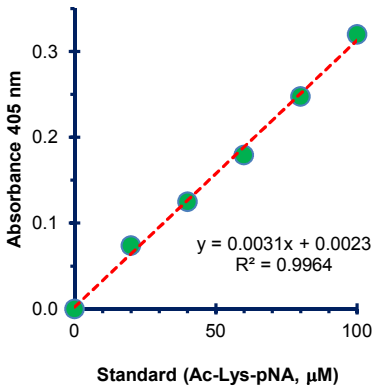
- Read the entire protocol before beginning the procedure.
- The HeLa nuclear extract and Lysine Developer should be refreeze immediately at –20 or -70°C after each use to avoid loss of activity.
- If positive and negative controls are designed, the kit provides sufficient reagents for 5 positive control assays with the HeLa Nuclear Extract and 5 Negative Control assays with the HDAC Inhibitor, Trichostatin A.
- Using 96-well plates with U-shape bottom. Flat bottom may give a little low value.

## IV. HDAC 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.

## V. 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 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 into a set of wells on the microtiter plate. Use 90 µl of $H_2O$ 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 ΔO.D./µM.
6. Based on the slope, you can determine the absolute amount of deacetylated lysine generated in your sample.



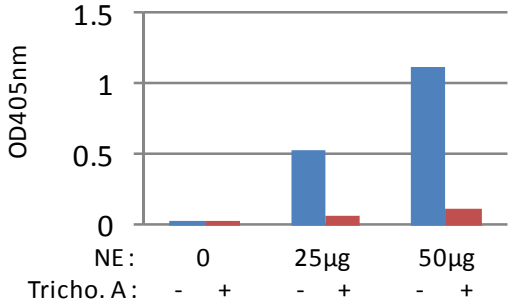




**Fig. HDAC Activity Assay:** Different amount of nuclear extract (NE) were tested following kit protocol in the presence and absence of HDAC Inhibitor (Incubated for 4 hrs).

## RELATED PRODUCTS:

| | |
|---|---|
| HDAC Fluorometric Assay kit | HDAC Drug Screening Kit |
| HDAc Inhibitors Set | HAT Activity Assay Kit |
| Histone H2A, H2B, H3 & H4 Antibodies | HDAC (1-11) Polyclonal Antibodies & Set |

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***FOR RESEARCH USE ONLY! Not to be used on humans.***

## GENERAL TROUBLESHOOTING GUIDE:

| Problems | Cause | Solution |
|---|---|---|
| Assay not working | • Use of ice-cold assay buffer | • Assay buffer must be at room temperature |
| | • Omission of a step in the protocol | • Refer and follow the data sheet precisely |
| | • Plate read at incorrect wavelength | • Check the wavelength in the data sheet and the filter settings of the instrument |
| | • Use of a different 96-well plate | • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates |
| Samples with erratic readings | • Use of an incompatible sample type | • Refer data sheet for details about incompatible samples |
| | • Samples prepared in a different buffer | • Use the assay buffer provided in the kit or refer data sheet for instructions |
| | • Cell/ tissue samples were not completely homogenized | • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope |
| | • Samples used after multiple free-thaw cycles | • Aliquot and freeze samples if needed to use multiple times |
| | • Presence of interfering substance in the sample | • Troubleshoot if needed |
| | • Use of old or inappropriately stored samples | • Use fresh samples or store at correct temperatures until use |
| Lower/ Higher readings in Samples and Standards | • Improperly thawed components | • Thaw all components completely and mix gently before use |
| | • Use of expired kit or improperly stored reagents | • Always check the expiry date and store the components appropriately |
| | • Allowing the reagents to sit for extended times on ice | • Always thaw and prepare fresh reaction mix before use |
| | • Incorrect incubation times or temperatures | • Refer datasheet & verify correct incubation times and temperatures |
| | • Incorrect volumes used | • Use calibrated pipettes and aliquot correctly |
| Readings do not follow a linear pattern for Standard curve | • Use of partially thawed components | • Thaw and resuspend all components before preparing the reaction mix |
| | • Pipetting errors in the standard | • Avoid pipetting small volumes |
| | • Pipetting errors in the reaction mix | • Prepare a master reaction mix whenever possible |
| | • Air bubbles formed in well | • Pipette gently against the wall of the tubes |
| | • Standard stock is at an incorrect concentration | • Always refer the dilutions in the data sheet |
| | • Calculation errors | • Recheck calculations after referring the data sheet |
| | • Substituting reagents from older kits/ lots | • Use fresh components from the same kit |
| Unanticipated results | • Measured at incorrect wavelength | • Check the equipment and the filter setting |
| | • Samples contain interfering substances | • Troubleshoot if it interferes with the kit |
| | • Use of incompatible sample type | • Refer data sheet to check if sample is compatible with the kit or optimization is needed |
| | • Sample readings above/below the linear range | • Concentrate/ Dilute sample so as to be in the linear range |
| **Note:** The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems. | | |