Product Information

HDAC Activity Fluorometric Assay Kit

I. Kit Contents:

Components	K2031-96 96 assays	Cap Color
HDAC Substrate	1 x 500 µl	Amber
10X HDAC Assay Buffer	1 x 1.0 ml	Green
Lysine Developer	1 x 1.0 ml	Orange
HDAC Inhibitor (Trichostatin A, 1 mM)	1 x 10 µl	Blue
HeLa Nuclear Extract (5 mg/ml)	1 x 10 µl	Red
Deacetylated Standard (4 mM)	1 x 20 µl	Yellow

II. Introduction:

Histone deacetylase (HDAC) is an enzyme that removes acetyl group from a histone and plays an important role in regulating gene expression. Inhibition of HDAC can regulate transcription and induce apoptosis or differentiation in cancer cells. However, screening compounds that inhibit HDAC is difficult due to the lack of convenient way for detecting HDAC activity.

The HDAC Activity Fluorometric Assay Kit provides a fast and convenient way for detection of HDAC activity based on fluorescence method that eliminates radioactivity, chromatography or extractions in traditional assays. The assay needs only two easy steps performed on the same microtiter plate. First, the HDAC substrate that contains an acetylated lysine side chain is incubated with the sample containing HDAC activity (e.g., HeLa nuclear extract). Second, Deacetylation of the HDAC substrate sensitizes the substrate, so that further treatment with the Lysine Developer generates a fluorophore. The fluorophore can be easily detected using a fluorometer or a fluorescence plate reader. The assay is well suitable for high throughput screening applications.

III. General Consideration:

Read the entire protocol before beginning the procedure.

The HeLa extract should be refrozen immediately at –70℃ after each use to avoid loss of activity.

After opening the kit, the Lysine Developer is recommended to be aliquoted and refreeze at –20℃ for future use.

If positive and negative controls are designed, the kit provides sufficient reagents for 5 positive control assays with the HeLa Nuclear Extra ct and 5 Negative Control assays with the HDAC Inhibitor, Trichostatin A.

IV. Histone Deacetylase Assay Protocol:

1. Dilute test samples (10 - 50 µg of nuclear extract or cell lysate) to 85 µl (final volume) of ddH_2O in each well (For background reading, add 85 µl ddH_2O only). For positive control, dilute 2 µl of HeLa nuclear extract with 83 µl ddH_2O. For negative control, dilute the sample into 83 µl of $ddH_2O$and then add 2 µl of Trichostatin A, 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℃ for 30 min (or longer if desired).
5. Stop the reaction by adding 10 µl of Lysine Developer and mix well. Incubate the plate at 37℃ for 30 min.

6. Read sample in a fluorescence plate reader (Ex/Em = 350 – 380/440 – 460 nm). The 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.

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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 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 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℃ 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 (Ex/Em = 350 - 380/440 - 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.

Analyses of HDAC Activity in HeLa Nuclear Extract. HeLa nuclear extract (NE) in various amounts were incubated with 5 µl HDAC fluorometric substrate. After 30 min, the reactions were stopped with 10 µl Lysine Developer. Samples were then read in a fluorescence plate reader with Ex/Em = 360/460 nm.

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the Nucleotide releasing buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly

Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • 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.		

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Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com