HDAC Activity Fluorometric Assay Kit

(Catalog #K330-100; 100 assays; Store kit at –80°C)

I. Introduction:

Inhibition of histone deacetylase (HDAC) has been implicated to modulate transcription and induce apoptosis or differentiation in cancer cells. However, screening compounds for HDAC inhibition has been difficult due to the lack of convenient tools for analyzing HDAC activity. The Fluorometric HDAC Activity Assay 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 substrate, which comprises an acetylated lysine side chain, is incubated with a sample containing HDAC activity (e.g., HeLa nuclear extract). Deacetylation of the substrate sensitizes the substrate, so that further 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. HDAC inhibitors and antibodies are also available separately.

II. Kit Contents:

Component	K330-100 100 assays	Cap Color	Part Number
HDAC Substrate	500 µl	Amber	K330-100-1
10X HDAC Assay Buffer	1.0 ml	Green	K330-100-2
Lysine Developer	1.0 ml	Orange	K330-100-3
HDAC Inhibitor (Trichostatin A, 1 mM)	10 µl	Blue	K330-100-4
HeLa Nuclear Extract (5 mg/ml)	10 µl	Red	K330-100-5
Deacetylated Standard (4 mM)	20 µl	Yellow	K330-100-6

III. General Consideration:

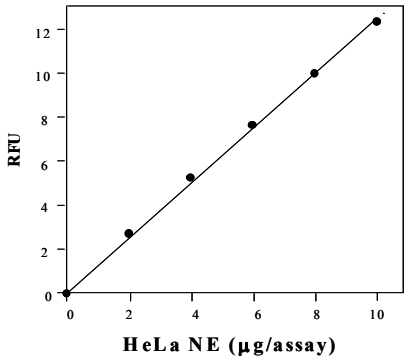
- Read the entire protocol before beginning the procedure.
- The HeLa extract should be refrozen immediately at –70°C after each use to avoid loss of activity.
- After opening the kit, the Lysine Developer is recommended to be aliquoted and refreeze at –20°C for future use.
- If positive and negative 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.

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°C 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°C 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.

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°C 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.

RELATED PRODUCTS:

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

This product has been manufactured by BioVision, Inc., under a license granted by Cyclex Co., Ltd. Under the following patents: **EP 1243658, US 7033778 & 7256013** and **JP4267043.**

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.		