InSitu HDAC Activity Fluorometric Assay Kit

rev. 03/16

(Catalog # K339-100; 100 assays; Store at -20°C)

I. Introduction:

Histone acetylases (HAT's) and Histone deacetylases (HDAC's) are associated with regulation of gene expression. In general, increased levels of histone acetylation are associated with increased transcriptional activity, whereas decreased levels of acetylation are associated with repression of gene expression. HDAC's are localized in both the cytosol and nucleus and some shuttle between the nucleus and cytosol. Increased HDAC expression has been observed in various cancers. BioVision's *InSitu* HDAC Activity Fluorometric Assay Kit provides a direct, fast, fluorescence-based method to measure the *InSitu* HDAC activity. The procedure requires two easy steps, all performed in the same cell culture plate. First, the cell permeable HDAC Substrate, which comprises an acetylated lysine side chain, is incubated with cells grown in a 96-well plate. Inside the cells, HDAC deacetylates the substrate. The second step involves lysing the cells and treating with the Developer that produces a fluorophore from the Deacetylated HDAC Substrate. The generated fluorescence can be quantified at Ex/Em = 368/442 nm. The assay is well suited for high throughput screening applications.

Step 1: Cells grown in 96-well plate + HDAC Substrate in medium $\xrightarrow{\text{Incubate for 1-3 hrs}}$ Deacetylated Substrate (within the cells)

Step 2: Deacetylated Substrate + Developer $\xrightarrow{\text{Incubate at 37°C for 30 min}}$ Fluorophore (Ex/Em = 368/442 nm)

II. Application:

- Measurement of HDAC activity in cells in 96-well format
- Screening HDAC inhibitors or activators
- Studying growth factors or other regulators that influence HDAC activity

III. Sample Type:

- Cultured adherent or suspension cells

IV. Kit Contents:

Components	K339-100	Cap Code	Part Number
HDAC Assay Buffer	25 ml	WM	K339-100-1
HDAC Substrate	100 µl	Amber	K339-100-2
Developer	1 ml	Orange	K339-100-3
HDAC Inhibitor (Trichostatin A [TSA], 1 mM)	10 µl	Blue	K339-100-4
Positive Control (Jurkat Cell Lysate)	Lyophilized	Violet	K339-100-5
Standard (Deacetylated Substrate, 4 mM)	100 µl	Yellow	K339-100-6

V. User Supplied Reagents and Equipment:

- Tissue culture treated 96-well plate. Clear or black plate with flat clear bottom.
- Multi-well spectrophotometer capable of reading fluorescence (ELISA reader).
- Phosphate Buffered Saline (PBS)

VI. Storage and Handling:

Store kit at –20°C, protected from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.

VII. Reagent Preparation and Storage Conditions:

- **HDAC Assay Buffer:** Store at -20°C or 4°C. Briefly warm at 37°C before use.
- **HDAC Substrate:** Aliquot & store at -20°C. Avoid repeated freeze-thaw. **Note:** Use a fresh pipette tip each time.
- **Developer:** Aliquot & store at -20°C. Avoid repeated freeze-thaw. Keep on ice while in use. Use within 2 months.
- **HDAC Inhibitor (Trichostatin A):** Aliquot & store at –20°C. Avoid repeated freeze-thaw.
- **Positive Control:** Reconstitute with 25 µl deionized water just prior to use. Mix gently by pipetting. Aliquot and store at -80°C. Avoid repeated freeze-thaw. Use within 2 months. Store lyophilized Positive Control at -20°C.
- **Standard (Deacetylated Substrate):** Store at –20°C.

VIII. *InSitu* HDAC Assay Protocol:

1. **Sample Preparation:** Seed 10^4 to 10^5 cells per well in a 96 well cell culture plate, in an appropriate medium. Treat log phase cells with desired method. Seed additional sample well(s) in parallel as the inhibitor control. For background control, use empty well(s) without cells.
2. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. Ensure thorough mixing prior to adding to the cells. For each well, prepare a 100 µl mix containing:

	Reaction Mix	Inhibitor Control Mix
Cell culture media	99 µl	98 µl
HDAC Substrate	1 µl	1 µl
HDAC Inhibitor	----	1 µl

Remove the old media and add 100 µl of the Reaction Mix to each well containing the test samples & background control and 100 µl of inhibitor control mix to each well containing the sample inhibitor control. **Note:** for suspension cells, spin down the cells at 1000 g for 5 minutes. Remove media & add the Reaction Mix.

3. **Positive Control:** Add 2 µl of positive control and 1 µl of HDAC Substrate to the desired well(s) and adjust the volume to 100 µl with Phosphate Buffered Saline (not provided).
4. **Incubation:** Incubate for one, two or three hours under cell culture conditions. **Note:** Incubation time depends on the HDAC activity of the sample.
5. **Standard Curve:** To generate 0, 200, 400, 600, 800 and 1000 pmol/well of Deacetylated Substrate Standard, dilute the Standard to 100 µM by adding 2.5 µl of 4 mM Standard to 97.5 µl of HDAC Assay Buffer and mix well. Add 0, 2, 4, 6, 8 and 10 µl of the diluted 100 µM Standard into a series of wells in a 96-well plate. Adjust the volume to 100 µl with HDAC Assay Buffer. The Standard Curve is linear up to 5000 pmol/well of Deacetylated Substrate Standard.
6. **Developer:** Mix enough reagents for the number of assays to be performed. For each well, prepare 100 µl Developer Mix containing:

	Developer Mix
Developer	10 µl
HDAC Assay Buffer	90 µl

Add 100 µl Developer Mix to each well containing Standard, test samples & controls. Incubate for 30 minutes at 37°C.

7. **Measurement:** Read fluorescence at Ex/Em = 368/442 nm.
8. **Calculation:** Subtract the 0 Standard reading from all Standard readings. Plot the Deacetylated Substrate Standard Curve. Correct sample background by subtracting the value derived from the background control from all sample readings. Apply the corrected sample reading to the Standard curve to get B pmol of Deacetylated Substrate in the sample wells.

Sample HDAC activity = B/T = pmol/min = mU

Where: **B** is the deacetylated substrate amount from Standard Curve (pmol)
T is the reaction time (min)

Unit Definition: One unit of HDAC is the amount of enzyme that generates one nanomole (1000 pmol) of deacetylated substrate/min at 37°C.

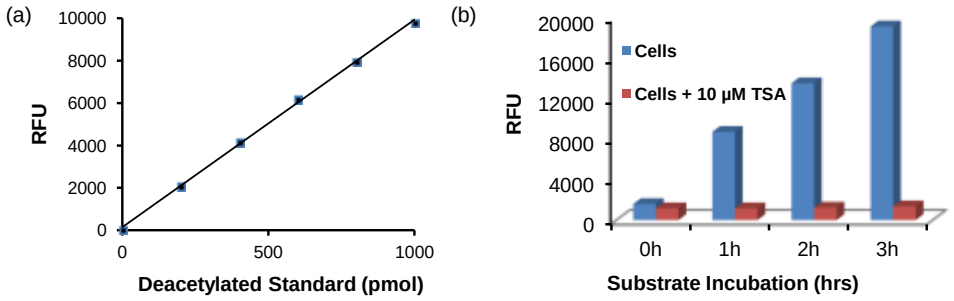

Figure: Deacetylated Substrate Standard Curve (a). *InSitu* HDAC Activity in HeLa Cells (b). Assays were performed following kit protocol. RFU is relative fluorescence units.

IX. RELATED PRODUCTS:

HDAC1 - HDAC11 Antibodies
DiscoveryPak™ HDAC Inhibitor Set
HDAC Fluorometric Activity Assay Kit
HAT Activity Colorimetric Assay Kit
HAT1 - HAT3 Antibodies
HDAC3 Inhibitor Screening Kit
HDAC8 Activity Assay Kit
HDAC8, human recombinant
HDAC Family Antibody Set
HDAC Colorimetric Activity Assay Kit
HDAC Inhibitor Drug Screening Kit
HAT (P/CAF) Active Human Recombinant
HDAC3 Activity Assay Kit
HDAC3, human recombinant
HDAC8 Inhibitor Screening Kit
Trichostatin A

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