HDAC Cell-Based Assay Kit

Catalog Number KA1319

96 assays

Version: 03

Intended for research use only

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Introduction

Background

Nucleosomes, which fold chromosomal DNA, contain two molecules each of the core histones H2A, H2B, H3, and H4. Almost two turns of DNA are wrapped around this octameric core, which represses transcription.[1] The histone amino termini extend from the core, where they can be modified post-translationally by acetylation, phosphorylation, ubiquitination, and methylation, affecting their charge and function.

Acetylation of the **ε**-amino groups of specific histone lysines is catalyzed by histone acetyltransferases (HATs) and correlates with an open chromatin structure and gene activation. Histone deacetylases (HDACs) catalyze the hydrolytic removal of acetyl groups from histone lysine residues and correlates with chromatin condensation and transcriptional repression.[2,3] Therefore, HDAC inhibition results in transcriptional activation through the conformational relaxation of DNA. Changes in the transcription of key genes has linked HDAC inhibitors to blocking angiogenesis and cell cycling, and promoting apoptosis and differentiation. By targeting these key components of tumor proliferation, HDAC inhibitors are currently being explored as potential anticancer agents.[4-6]

Principle of the Assay

The HDAC Cell-Based Assay Kit provides an easy tool for studying HDAC activity modulators in whole cells. By using a cell-permeable HDAC substrate, the activity of various protein lysine-specific deacetylases including HDAC1-containing complexes can be measured in intact cells in a simple and homogenous manner. The fluorescence of the deacetylated reaction product can be analyzed using a plate reader or a fluorometer with excitation wavelengths of 340-360 nm and emission wavelengths of 440-465 nm. An HDAC inhibitor, trichostatin A, is included for checking specificity of the HDAC reaction.

General Information

Materials Supplied

List of component

Item	Amount
HDAC Assay Buffer (10X)	5 mL
Nonidet P-40 Assay Reagent (10%)	500 µL
HDAC Deacetylated Standard	400 µL
HDAC1 Positive Control	50 µL
Cell-Based Assay HDAC Substrate	100 µL
HDAC Developer	20 mg
HDAC Trichostatin A	250 µL
96-Well Clear Bottom Black Culture Plate	1 plate

Storage Instruction

The kit will perform as specified if components are stored as directed below and used before the expiration date indicated on the outside of the box. For best results, remove components and store as stated below.

Item	Storage
HDAC Assay Buffer (10X)	-20°C
Nonidet P-40 Assay Reagent (10%)	4°C
HDAC Deacetylated Standard	-20°C
HDAC1 Positive Control	-80°C
Cell-Based Assay HDAC Substrate	-20°C
HDAC Developer	-20°C
HDAC Trichostatin A	-20°C
96-Well Clear Bottom Black Culture Plate	Room Temperature

Materials Required but Not Supplied

- ✓ Cell line such as HeLa cells or MCF-7 cells (can be obtained from ATCC); other cell lines may also be used
- ✓ A fluorometer with the capacity to measure fluorescence using an excitation wavelength of 340-360 nm and an emission wavelength of 440-465 nm
- ✓ Adjustable pipettes and a repeat pipettor
- ✓ A source of pure water; glass distilled water or HPLC-grade water is acceptable

Precautions for Use

Warning: This product is for research only - Not for human or veterinary diagnostic or therapeutic use.

✓ Precautions

Please read these instructions carefully before beginning this assay.

✓ Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Material Safety Data Sheet, which has been sent via email to your institution.

✓ Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Assay Protocol

Reagent Preparation

- ✓ HDAC Assay Buffer (10X)

  Dilute 5 mL of HDAC Assay Buffer (10X) with 45 mL of UltraPure water. This final Assay Buffer should be used for diluting the HDAC Deacetylated Standard, HDAC1 Positive Control, Cell-Based Assay HDAC Substrate, and for dissolving the HDAC Developer. The diluted buffer is stable for six months at -20°C.

- ✓ HDAC Deacetylated Standard

  The HDAC Deacetylated Standard vial contains 400 µL of 2.1 mM deacetylated standard in dimethylsulfoxide (DMSO). The deacetylated standard is used to prepare a standard curve for quantitative determination of HDAC activity.

  To run a standard curve, obtain six clean test tubes and label them #1 through #6. Add 1.4 mL of Assay Buffer into tube #1 and 500 µL into tubes #2-6. Transfer 20 µL of the HDAC Deacetylated Standard to tube #1 and mix thoroughly. The concentration of this standard, the first point on the standard curve, is 30 µM. Serially dilute the standard by removing 500 µL from tube #1 and placing it into tube #2; mix thoroughly. Next remove 500 µL from tube #2 and place it into tube #3; mix thoroughly. Repeat the procedure for tubes #4-5. The concentration of these standards will be 30, 15, 7.5, 3.75, and 1.88 µM, respectively. Do not add any standard to tube #6. This tube will be your blank.

- ✓ HDAC1 Positive Control

  The HDAC1 Positive Control vial contains 50 µL of human recombinant HDAC1. Dilute 10 µL of the control with 190 µL of diluted Assay Buffer. The diluted HDAC1 is stable for four hours when stored on ice.

- ✓ HDAC Trichostatin A

  The HDAC Trichostatin A vial contains 250 µL of 0.21 mM Trichostatin A. Trichostatin A is an HDAC inhibitor. Dilute 50 µL of Trichostatin A stock with 450 µL of diluted Assay Buffer. A 10 µL aliquot in the assay results in a final concentration of 2.1 µM. At this concentration, HDAC activity will be completely inhibited.

- ✓ Cell-Based Assay HDAC Substrate

  The Cell-Based Assay HDAC Substrate vial contains 100 µL of concentrated Boc-Lys(AC)-AMC in DMSO. Prior to assaying, dilute 100 µL of the Substrate with 1 mL of diluted Assay Buffer.

- ✓ HDAC Lysis/Developer Mixture

  NOTE: this HDAC Lysis/Developer Mixture should be prepared immediately before use.

  To one vial of Developer, add 4.5 mL of diluted Assay Buffer, 72 µL of HDAC Trichostatin A, and 500 µL of Nonidet P-40 Assay Reagent (10%).

Sample Preparation

✓ Cell Culture Preparation

- Seed cells in the provided clear bottom black 96-well plate at a density of (2×10^4) - (5×10^4) cells/well in 100 µL of culture medium. For a blank control, add 100 µL of culture medium without cells to 2-3 wells of the plate.

  NOTE: If you run a standard curve with the assay, add 100 µL of culture medium without cells to 12 wells of the plate. Culture the cells overnight or until the cells reach 80% confluence.
- Treat the cells with or without compounds to be tested. We recommend that each treatment be performed in duplicate.
- Following addition of test compounds, continue to culture the cells in a CO_2 incubator at 37°C for 24-48 hours, or for a period of time according to your typical experimental protocol.

Assay Procedure

✓ Plate Set Up

There is no specific pattern for using the wells on the plate. However, a positive control in duplicate has to be assayed with the sample. We suggest that each sample be assayed at least in duplicate and to have two wells designated as background wells. We also recommend assaying each sample in the presence and absence of the HDAC inhibitor to allow for the correction of HDAC-independent fluorescence. Record the contents of each well on the template sheet provided in Plate Layout.

1. Centrifuge the plate in a plate centrifuge at 500 x g for five minutes.
2. Aspirate the culture medium.
3. Add 200 µL of diluted Assay Buffer to each well and centrifuge the plate at 500 x g for five minutes.
4. Aspirate the supernatant.
5. Add 90 µL of culture medium or positive control to non-inhibited sample wells. Add 80 µL of culture medium plus 10 µL of Trichostatin A to appropriate control wells to test assay specificity. Initiate the HDAC reactions by adding 10 µL of diluted HDAC Substrate to each well.
6. Incubate the plate at 37°C for two hours for optimal development (can be 1-3 hours according to your schedule).
7. Add 100 µL standards prepared above to appropriate wells. Skip this step if you are not running a standard curve with the assay.
8. Add 50 µL of the Lysis/Developer Solution to each well.
9. Shake the plate on a plate shaker for a 1-2 minutes.
10. Incubate the plate for 15 minutes at 37°C.
11. Read the fluorescent intensity of each well (excitation = 340-360 nm; emission = 440-460 nm).

Data Analysis

Calculation of Results

✓ Plot the Standard Curve

1. Determine the average fluorescence of the standards. Subtract the fluorescence value of the blank (standard tube #6) from itself and all other standards. This is the corrected fluorescence.
2. Plot the corrected fluorescence values (from step 1 above) of each standard as a function of the final concentration of Deacetylated Standards. See Figure 1, for a typical standard curve.

✓ Determination of HDAC activity

1. Determine the average fluorescence of each sample and sample plus Trichostatin A.
2. Subtract the Trichostatin A sample fluorescence from the non-Trichostatin A sample fluorescence to yield the corrected sample fluorescence.
3. Calculate the HDAC activity using the equation obtained from the linear regression of the standard curve, substituting corrected fluorescence values for each sample.

$$\text{HDAC Activity (nmol/min/ml)} = \frac{[\text{Corrected Sample Fluorescence} - (\text{y-intercept})/\text{slope}] \times \text{dilution}}{15\ \text{minutes}}$$

If you anticipate a high activity of HDAC in the samples, dilution may be required to obtain values that fall on the standard curve.

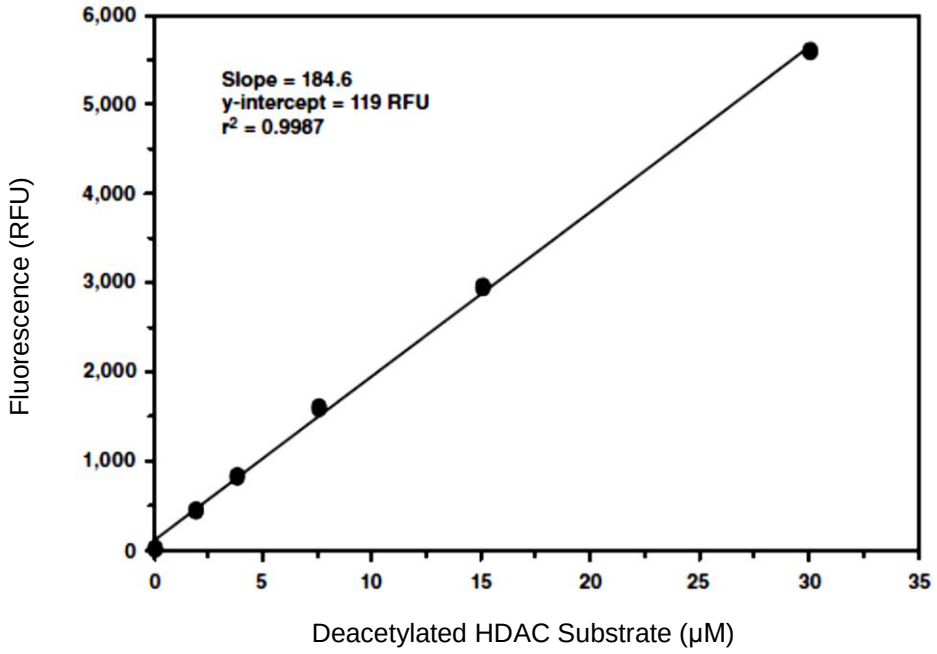

Figure 1. Deacetylated substrate standard curve

Performance Characteristics

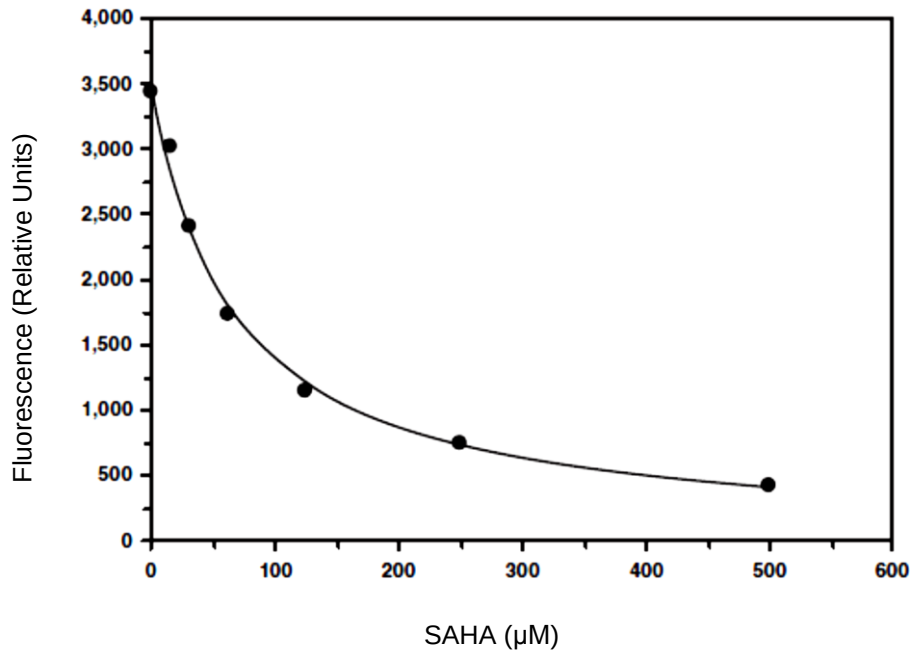

Figure 2. Inhibition of HDAC activity by SAHA. MCF-7 cells were seeded in a 96-well clear bottom black plate at a density of 4 x 10^4 cells/well. The next day, cells were treated with different concentrations of SAHA as indicated above. On the third day, cells were processed for the measurement of HDAC activity according to the protocol described in this booklet.

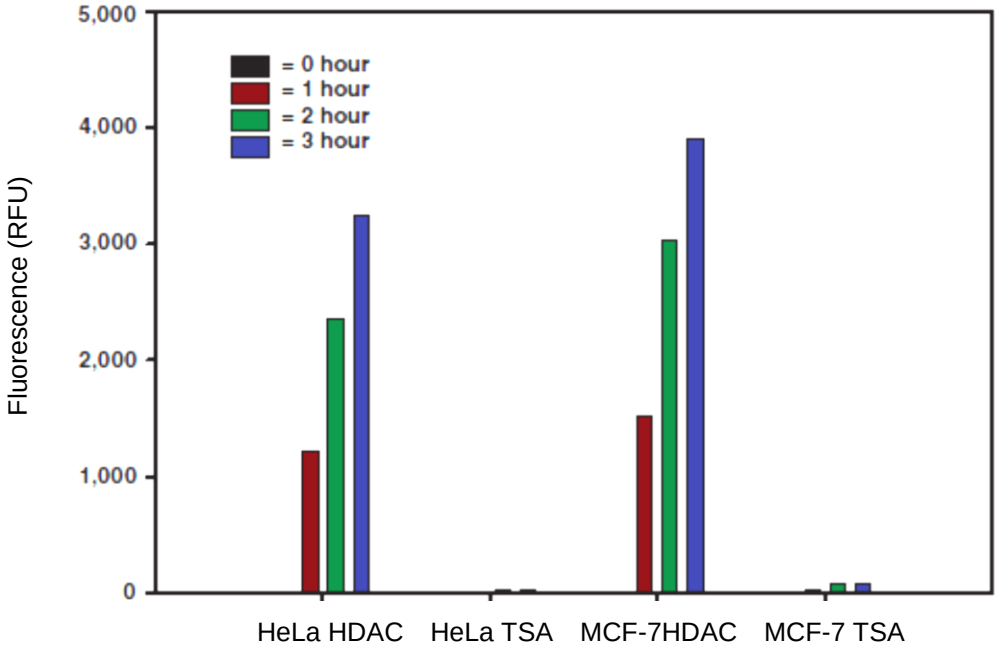

Figure 3. Effect of substrate incubation time on HDAC activity in HeLa cells and MCF-7 cells. HeLa cells and MCF-7 cells were seeded in a 96-well clear bottom black plate at a density of 4 x 10^4 cells/well. The next day, cells were processed for the measurement of HDAC activity according to the protocol described in this booklet. One hour of substrate incubation generates measurable fluorescence intensity whereas two hours of substrate incubation time doubles the amount of fluorescence activity indicating linearity of the assay over this time period. Addition of TSA almost completely blocks fluorescence, indicating specificity of the HDAC assay.

Resources

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersions of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
Erratic response curve of compound treatments	Unequal number of cells in each well	Make sure each well contains the same number of cells
The fluorometer exhibited 'MAX' values for the wells	The GAIN setting is too high	Reduce the GAIN and re-read
High reading in all wells	Cell density is too high	Plate cells more sparsely

References

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Plate Layout

12								
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7								
6								
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4								
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2								
1								
	A	B	C	D	E	F	G	H