Cayman CHEMICAL

# HDAC Activity/Inhibitor Screening Assay Kit

*Catalog No. 789701*

## TABLE OF CONTENTS


Contents of the Kit ..... 1
Precautions and Warnings ..... 2
Warranty and Limitations of Remedy ..... 2
If You Have Problems ..... 2
Storage and Stability ..... 2
Additional Items Required ..... 3
About this Assay ..... 3
Precision and Sensitivity ..... 3
Pre-Assay Preparation ..... 3
Sample Preparation ..... 4
Performing the Activity Assay ..... 5
Calculating HDAC Activity ..... 6
Performing the Inhibitor Screening Assay ..... 7
Calculating the Percent Inhibition ..... 8
Interferences ..... 9
Troubleshooting ..... 9
References ..... 10
Related Products ..... 10
Plate Template ..... 10
Notes ..... 10

## CONTENTS OF THE KIT*

| Number | Item | Quantity |
|---|---|---|
| 1 | HDAC Assay Buffer (10X) | 1 vial |
| 2 | HDAC HeLa Nuclear Extract | 1 vial |
| 3 | HDAC Trichostatin A | 1 vial |
| 4 | HDAC Substrate | 1 vial |
| 5 | HDAC Deacetylated Standard | 1 vial |
| 6 | HDAC Developer | 2 vials |
| 7 | 96 Well Black Plate | 1 plate |
| 8 | Plate Cover | 1 cover |

If any of the items listed above are damaged or missing, please contact our Customer Service department at (888) 526-5351 or (734) 975-3999. We cannot accept any returns without prior authorization.

**Sold under license from Cyclex.*

## PRECAUTIONS & WARNINGS

**WARNING: THIS PRODUCT IS NOT INTENDED OR APPROVED FOR USE IN HUMANS OR VETERINARY ANIMALS. RELIANCE ON THIS PRODUCT FOR ANALYTE MEASUREMENTS IN A THERAPEUTIC SETTING IS HAZARDOUS AND MAY RESULT IN ILLNESS OR INJURY.**

1. Read these instructions carefully before beginning the assay.
2. For research use only. Not for human or diagnostic use.

## WARRANTY AND LIMITATION OF REMEDY

Cayman Chemical Company makes no warranty or guarantee of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman warrants only to the original customer that the material will meet our specifications at the time of delivery.

Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have any obligation or liability, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence.

This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's exclusive remedy and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.

## IF YOU HAVE PROBLEMS

Our technical service staff may be reached by phone (888-526-5351, 734-975-3888), fax (734-971-3641), or E-Mail (techserv@caymanchem.com) Monday through Friday 8:00 AM to 6:00 PM EST. In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

## STORAGE AND STABILITY

This kit will perform as specified if stored at -80°C and used before the expiration date indicated on the outside of the box.

## ADDITIONAL ITEMS REQUIRED

1. A fluorometer with the capacity to measure fluorescence using excitation wavelengths of 340-360 nm and emission wavelengths of 440-465 nm.
2. Adjustable pipettes and a repeat pipettor.
3. A source of UltraPure water (Milli-Q or HPLC-grade water).

## ABOUT THIS ASSAY

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]

The Cayman Chemical HDAC Activity/Inhibitor Screening Assay Kit provides a fast, fluorescence-based method for measuring HDAC activity that eliminates radioactivity, extraction, or chromatography. The procedure requires only two easy steps, both performed in the same microplate. In the first step, an acetylated lysine substrate is incubated with samples containing HDAC activity. Deacetylation sensitizes the substrate such that treatment with the HDAC developer in the second step releases a fluorescent product. The fluorophore can be easily analyzed using a fluorescence plate reader or a fluorometer with excitation wavelengths of 340-360 nm and emission wavelengths of 440-465 nm. The assay can be used for quantifying Class I and II HDAC activity from various sources or for the identification of HDAC inhibitors in high throughput screening programs.

## PRECISION AND SENSITIVITY

**Precision:** Intra-assay coefficient of variation = 3.9% (n = 84). Inter-assay coefficient of variation = 4.1% (n = 6).

**Sensitivity:** Under the standardized conditions of the assay described in this booklet, the dynamic range of the kit is 0-118 nmol/min/ml of HDAC activity.

## PRE-ASSAY PREPARATION

**Preparation of reagents**

1. **HDAC Assay Buffer (10X) - (vial #1)**
   Dilute 5 ml of Assay Buffer concentrate with 45 ml of UltraPure water. This final Assay Buffer [25 mM Tris-HCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, and 1 mM $MgCl_2$] should be used in the assay. It is used for diluting the HeLa Nuclear Extract, samples, deacetylated standard, and for dissolving the HDAC developer. The diluted buffer is stable for 6 months at 4°C.

2. **HDAC HeLa Nuclear Extract - (vial #2)**
   The vial contains 450 µl of nuclear extract from HeLa cells (human cervical cancer cells). To avoid repeated freezing and thawing, the nuclear extract should be aliquoted into several small vials and refrozen at -80°C. If using the HeLa nuclear extract as a positive control in the activity assay, dilute 30 µl of the extract with 60 µl of diluted assay buffer. If using the extract for inhibitor screening, dilute 350 µl of nuclear extract with 700 µl of diluted assay buffer. The diluted HeLa nuclear extract is stable for 4 hours when stored on ice.

3. **HDAC Trichostatin A - (vial #3)**
   The vial contains 250 µl of 0.21 mM Trichostatin A. Trichostatin A is a HDAC inhibitor. If performing the activity assay, 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 1 µM. At this concentration, HDAC activity will be completely inhibited.

4. **HDAC Substrate - (vial #4)**
   The vial contains 1.2 ml of 3.4 mM acetylated fluorometric substrate in dimethylsulfoxide. The solution is ready to use as supplied. *NOTE: The $K_m$ value for the HDAC substrate is 27.5 µM for the HeLa nuclear extract. The final concentration of HDAC substrate in the activity and inhibitor screening assays, as described below, is 200 µM. This concentration may be reduced by dilution with dimethylsulfoxide at the user's discretion, particularly when assaying for competitive inhibitors.*

5. **HDAC Deacetylated Standard - (vial #5)**
   The vial contains 400 µl of 2.1 mM deacetylated standard in dimethylsulfoxide. The deacetylated standard is used to prepare a standard curve for quantitative determination of HDAC activity. It is not necessary to prepare a standard curve if screening for HDAC inhibitors.

**Preparation of deacetylated standards** - Dilute 200 µl of standard with 1.8 ml of assay buffer to obtain the stock solution. Take six clean glass test tubes and mark them A-F. Add the amount of standard stock and assay buffer to each tube as described in Table 1 (see below). Diluted standards are stable for 4 hours at room temperature.

| Tube | Deacetylated standard stock (µl) | Assay buffer (µl) | Deacetylated standard conc. (µM) |
|---|---|---|---|
| A | 0 | 1,000 | 0 |
| B | 50 | 950 | 10.5 |
| C | 100 | 900 | 21 |
| D | 200 | 800 | 42 |
| E | 400 | 600 | 84 |
| F | 800 | 200 | 168 |

*Table 1.*

6. **HDAC Developer - (vial #6)**
   The vial contains the HDAC developer. Dissolve the contents of the vial in 4 ml of diluted assay buffer and store on ice. Add 100 µl of Trichostatin A (vial #3) to the reconstituted developer. One vial of developer will develop the entire plate. The reconstituted developer is stable for 2 hours.

## SAMPLE PREPARATION

**Isolation of Cellular Nuclear Extract**

1. **Isolation of Nuclei**
   a) Suspend 1 x $10^7$ cells (100 mm dish sub-confluent) in 1 ml of cold lysis buffer (10 mM Tris-HCl, pH 7.5, 10 mM NaCl, 15 mM $MgCl_2$, 250 mM sucrose, 0.5% NP-40, and 0.1 mM EGTA).
   b) Vortex for 10 seconds and keep on ice for 15 minutes.
   c) Spin the cells through 4 ml of cold sucrose cushion (30% sucrose, 10 mM Tris-HCl, pH 7.5, 10 mM NaCl, and 3 mM $MgCl_2$) at 1,300 x g for 10 minutes at 4°C.
   d) Discard the supernatant.
   e) Resuspend the nuclei pellet in 1 ml of cold 10 mM Tris-HCl, pH 7.5, containing 10 mM NaCl.
   f) Centrifuge at 1,300 x g for 10 minutes at 4°C.
   g) Discard the supernatant.

2. **Extraction of Nuclear components**
   a) Suspend the isolated nuclei in 100-200 µl of extraction buffer (50 mM HEPES, pH 7.5, containing 420 mM NaCl, 0.5 mM EDTA, 0.1 mM EGTA, and 10% glycerol).
   b) Sonicate for 30 seconds and incubate on ice for 30 minutes.
   c) Centrifuge at 10,000 x g for 10 minutes at 4°C in a microcentrifuge.
   d) The supernatant contains the crude nuclear extract.
   e) Store the crude nuclear extract at -80°C until use.

### Pipetting Tips

It is recommended that a repeating pipettor be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times. Before pipetting each reagent, equilibrate the pipette tip in that reagent (i.e., fill the tip and expel the contents; repeat several times). Do not expose the pipette tip to the reagent(s) already in the well.

## PERFORMING THE ACTIVITY ASSAY

### Plate configuration

There is no specific pattern for using the wells on the plate. A typical layout of deacetylated standards and samples to be measured in duplicate is given below (see Figure 1). We suggest you record the contents of each well on the template sheet provided (see page 10).

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | A | A | 1 | 1 | 5 | 5 | 9 | 9 | 13 | 13 | 17 | 17 |
| B | B | B | 1T | 1T | 5T | 5T | 9T | 9T | 13T | 13T | 17T | 17T |
| C | C | C | 2 | 2 | 6 | 6 | 10 | 10 | 14 | 14 | 18 | 18 |
| D | D | D | 2T | 2T | 6T | 6T | 10T | 10T | 14T | 14T | 18T | 18T |
| E | E | E | 3 | 3 | 7 | 7 | 11 | 11 | 15 | 15 | 19 | 19 |
| F | F | F | 3T | 3T | 7T | 7T | 11T | 11T | 15T | 15T | 19T | 19T |
| G | HL | HL | 4 | 4 | 8 | 8 | 12 | 12 | 16 | 16 | 20 | 20 |
| H | HT | HT | 4T | 4T | 8T | 8T | 12T | 12T | 16T | 16T | 20T | 20T |

A-F - Standards A-F
HL - HeLa Nuclear Extract
HT - HeLa Nuclear Extract + Trichostatin A
1-20 - Samples 1-20
1T-20T - Samples 1-20 + Trichostatin A

*Figure 1. Sample Plate Format*

- The final volume of the assay is 210 µl in all the wells.
- Use the diluted assay buffer in the assay.
- All reagents except HeLa nuclear extract, samples, and developer must be equilibrated to room temperature before beginning the assay, (when performing the assay at 37°C, equilibrate the assay buffer to 37°C).
- It is not necessary to use all the wells on the plate at one time, however a standard curve should be run every time.
- If the HDAC activity of the sample is not known or if it is expected to be beyond the range of the standard curve, it is prudent to assay the sample at several dilutions.
- It is recommended that the samples and deacetylated standards be assayed at least in duplicate (triplicate recommended).
- Twelve samples in triplicate or twenty samples in duplicate can be run in the assay.
- Assay temperature can be room temperature or 37°C. Assaying at 37°C will increase HDAC activity and is recommended if samples have low HDAC activity.
- Monitor the fluorescence on a fluorometer with an excitation wavelength of 340-360 nm and an emission wavelength of 440-465 nm.

1. **Deacetylated Standard Wells** - add 150 µl of assay buffer and 10 µl of standard (tubes A-F) per well in the designated wells on the plate (see suggested plate configuration, Figure 1, above).
2. **HeLa Nuclear Extract Wells (positive control)** - add 140 µl of assay buffer and 10 µl of diluted HeLa nuclear extract to four wells.
3. **Sample Wells** - add 140 µl of assay buffer and 10 µl of sample to four wells. To obtain reproducible results, HDAC activity must fall within the standard curve. When necessary, samples can be diluted with assay buffer to bring the activity to this level.
4. Add 10 µl of diluted Trichostatin A to two of the HeLa nuclear extract wells and to two of each of the sample wells. Trichostatin A will eliminate all HDAC activity and is used as a control for generating the sample background values. Add 10 µl of assay buffer to the HeLa nuclear extract and samples wells that were not treated with Trichostatin A.
5. Initiate the reactions by adding 10 µl of HDAC substrate to all the wells being used including the standard wells. The final concentration of substrate is 200 µM in the wells.

6. Cover the plate with the plate cover and incubate on a shaker for 30 minutes at room temperature or 37°C.
7. Remove the plate cover and add 40 µl of developer. Cover the plate with the plate cover and incubate for 15 minutes at room temperature.
8. Remove the plate cover and read fluorescence using an excitation wavelength of 340-360 nm and an emission wavelength of 440-465 nm. It may be necessary to adjust the gain setting on the instrument to allow for the measurement of all the samples. The development is stable for 30 minutes.

## CALCULATING HDAC ACTIVITY

1. Calculate the average fluorescence of each standard, sample, and Trichostatin-treated sample.
2. Subtract the average fluorescence of standard A from itself and all other standards.
3. Plot the corrected fluorescence of the standards (from step 2 above) as a function of the final concentration of deacetylated standard from Table 1 (See page 4). See Figure 2 for a typical standard curve.

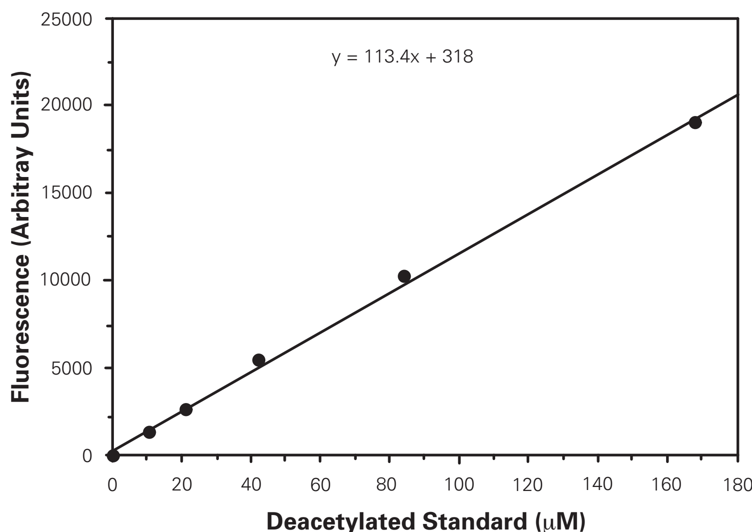


*Figure 2. Deacetylated standard curve*

4. Subtract the average fluorescence of the Trichostatin-treated samples from the average fluorescence of its corresponding samples to yield the corrected sample fluorescence (CSF).
5. Calculate the deacetylated concentration of the samples using the equation obtained from the linear regression of the standard curve substituting corrected sample fluorescence (CSF) values for each sample.

**Deacetylated compound (µM) = [(CSF – y-intercept)/slope]**

6. Calculate the HDAC activity using the following equation. One unit is defined as the amount of enzyme that will cause the formation of 1.0 nmol of deacetylated compound per minute at 25 or 37°C.

**HDAC Activity (nmol/min/ml) = [µM/30 minutes] x sample dilution**

## PERFORMING THE INHIBITOR SCREENING ASSAY

**Plate configuration**

There is no specific pattern for using the wells on the plate. However, it is necessary to have three wells designated as 100% initial activity and three wells designated as background wells. We suggest that each inhibitor sample be assayed in triplicate and that you record the contents of each well on the template sheet provided (See page 10). A typical layout of samples and inhibitors to be measured in triplicate is given below (see Figure 3).

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | BW | BW | BW | 7 | 7 | 7 | 15 | 15 | 15 | 23 | 23 | 23 |
| B | A | A | A | 8 | 8 | 8 | 16 | 16 | 16 | 24 | 24 | 24 |
| C | 1 | 1 | 1 | 9 | 9 | 9 | 17 | 17 | 17 | 25 | 25 | 25 |
| D | 2 | 2 | 2 | 10 | 10 | 10 | 18 | 18 | 18 | 26 | 26 | 26 |
| E | 3 | 3 | 3 | 11 | 11 | 11 | 19 | 19 | 19 | 27 | 27 | 27 |
| F | 4 | 4 | 4 | 12 | 12 | 12 | 20 | 20 | 20 | 28 | 28 | 28 |
| G | 5 | 5 | 5 | 13 | 13 | 13 | 21 | 21 | 21 | 29 | 29 | 29 |
| H | 6 | 6 | 6 | 14 | 14 | 14 | 22 | 22 | 22 | 30 | 30 | 30 |

BW - Background Wells
A - 100% Initial Activity Wells
1-30 - Inhibitor Wells

*Figure 3. Inhibitor Screening Plate Format*

- The final volume of the assay is 210 µl in all the wells.
- Use the diluted assay buffer in the assay.
- All reagents except HeLa nuclear extract and developer must be equilibrated to room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- If the appropriate inhibitor dilution is not known, it may be necessary to assay at several dilutions.
- We recommend assaying samples in triplicate, but it is the user's discretion to do so.
- Thirty inhibitor samples can be assayed in triplicate or forty-six in duplicate.
- The assay temperature is 22-25°C (room temperature).
- Monitor the fluorescence on a fluorometer with an excitation wavelength of 340-360 nm and an emission wavelength of 440-465 nm.

1. **100% Initial Activity Wells** - add 140 µl of assay buffer, 10 µl of diluted HeLa nuclear extract, and 10 µl of solvent (the same solvent used to dissolve the inhibitor) to three wells.
2. **Background Wells** - add 150 µl of assay buffer and 10 µl of solvent (the same solvent used to dissolve the inhibitor) to three wells.
3. **Inhibitor Wells** - add 140 µl of assay buffer, 10 µl of diluted HeLa nuclear extract, and 10 µl of inhibitor* to three wells.
4. Initiate the reactions by adding 10 µl of HDAC substrate to all the wells being used.
5. Cover the plate with the plate cover and incubate on a shaker for 30 minutes at room temperature.
6. Remove the plate cover and add 40 µl of developer. Cover the plate with the plate cover and incubate for 15 minutes at room temperature.
7. Remove the plate cover and read the fluorescence using an excitation wavelength of 340-360 nm and an emission wavelength of 440-465 nm. It may be necessary to adjust the gain setting on the instrument to allow for the measurement of all the samples. The development is stable for 30 minutes.

*Inhibitors can be dissolved in assay buffer, methanol, or dimethylsulfoxide and should be added to the assay in a final volume of 10 µl. In the event that the appropriate concentration of inhibitor needed for HDAC inhibition is completely unknown, we recommend that several dilutions of the inhibitor be assayed.

## CALCULATING THE PERCENT INHIBITION

1. Determine the average fluorescence of each sample.
2. Subtract the fluorescence of the background wells from all wells on the plate.
3. Determine the percent inhibition for each sample. To do this, subtract each inhibitor sample value from the 100% initial activity sample value. Divide the result by the 100% initial activity value and then multiply by 100 to give the percent inhibition.
4. Either graph the Percent Inhibition or Percent Initial Activity as a function of the inhibitor concentration to determine the $IC_{50}$ value (concentration at which there was 50% inhibition). An example of HeLa nuclear extract inhibition by Trichostatin A is shown in Figure 4, below.

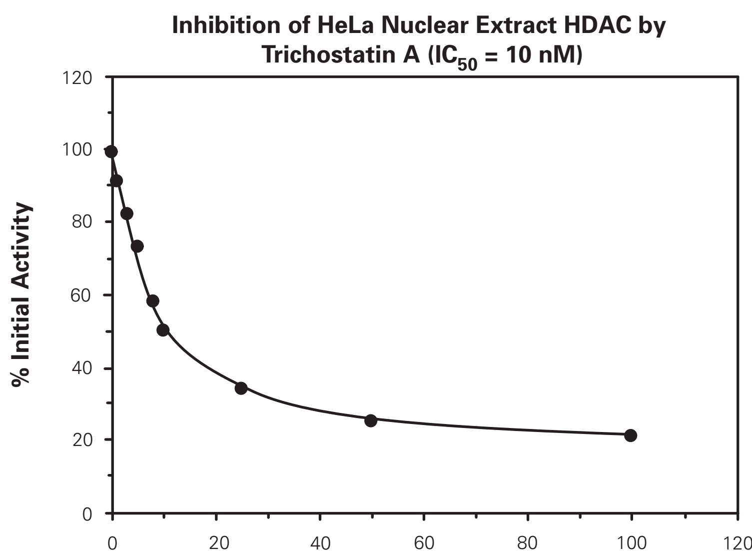


*Figure 4. Inhibition of HeLa nuclear extract by Trichostatin A ($IC_{50}$ = 10 nM; final concentration of HDAC substrate is 200 µM).*

## INTERFERENCES

The following reagents were tested for interference in the assay.

| | Reagent | Will Interfere (Yes or No) |
|---|---|---|
| **Buffers:** | Tris | No |
| | Borate | No |
| | HEPES | No |
| | Phosphate | No |
| **Protease Inhibitors/Chelators:** | PMSF (≤200 µM) | No |
| | Leupeptin (≤10 µg/ml) | No |
| | Pepstatin (≤10 µg/ml) | No |
| | Chymostatin (≤10 µg/ml) | No |
| | EGTA (≤1 mM) | No |
| | EDTA (≤1 mM) | No |
| **Solvents:** | Ethanol (10 µl) | Yes |
| | Methanol (10 µl) | No |
| | Dimethylsulfoxide (10 µl) | No |
| **Others:** | Glycerol (≤10%) | No |
| | Bovine serum albumin (≤1%) | No |
| | β-Mercaptoethanol (≤5 mM) | No |

## TROUBLESHOOTING

Problem: Erratic values; dispersion of duplicates/triplicates.
Cause: Poor pipetting/technique. *-or-* Bubble in the well(s).
Solution: Carefully tap the side of the plate with your finger to remove bubbles. Be careful not to splash the contents of the wells.

Problem: HDAC activity was not detected in the sample.
Cause: Sample was too dilute.
Solution: Re-assay the sample using a lower dilution.

Problem: Fluorescence value was at the maximal level in the sample wells.
Cause: The sample is too concentrated or the Gain setting is set too high.
Solution: Set the gain to a lower setting and measure the fluorescence. If the fluorescence is still too high, dilute your sample with diluted assay buffer and re-assay.

Problem: The deacetylated standard curve did not work.
Cause: Either the deacetylated standards were not diluted properly or the deacetylated standard has deteriorated.
Solution: Set-up the standards according to Table 1 and re-assay.

Problem: No inhibition seen with inhibitor.
Cause: The inhibitor concentration is not high enough. *-or-* The compound is not an inhibitor of the enzyme.
Solution: Increase the inhibitor concentration and re-assay.

## REFERENCES


1. Grunstein, M. Histone acetylation in chromatin structure and transcription. *Nature* **389,** 349-352 (1997).
2. Strahl, B.D. and Allis, D. The language of covalent histone modifications. *Nature* **403,** 41-45 (2000).
3. Cheung, W.L., Briggs, D.B., and Allis, C.D. Acetylation and chromosomal functions. *Curr. Opin. Cell Biol.* **12,** 326-333 (2000).
4. Kwon, H.J., Kim, M.S., Kim, M.J.*, et al.* Histone deacetylase inhibitor FK228 inhibits tumor angiogenesis. *Int. J. Cancer* **97,** 290-296 (2002).
5. Suenaga, M., Soda, H., Oka, M.*, et al.* Histone deacetylase inhibitors suppress telomerase reverse tarnscriptase mRNA expression in prostate cancer cells. *Int. J. Cancer* **97,** 621-625 (2002).
6. Johnstone, R.W. Histone-deacetylase inhibitors: novel drugs for the treatment of cancer. *Nature Reviews Drug Discover* **1**, 287-299 (2002).


## RELATED PRODUCTS

Trichostatin A - Cat. No. 89730 • CAY10398 - Cat. No. 89740 • CAY10433 - Cat. No. 10005019

## PLATE TEMPLATE

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | | | | | | | | | | | | |
| B | | | | | | | | | | | | |
| C | | | | | | | | | | | | |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

## NOTES

This document is copyrighted. All rights are reserved. This document may not, in whole or part, be copied, photocopied, reproduced, translated, or reduced to any electronic medium or machine-readable form without prior consent, in writing, from Cayman Chemical Company.

©03/20/2007, Cayman Chemical Company, Ann Arbor, MI, All rights reserved. Printed in U.S.A.