HDAC1 Inhibitor Screening Assay Kit

Catalog Number KA1320

96 assays

Version: 03

Intended for research use only

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Table of Contents

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

HDAC1 Inhibitor Screening Assay Kit provides a fast, fluorescent-based method for screening HDAC1 inhibitors. The procedure requires only two easy steps, both performed in the same microplate. In the first step, an acetylated lysine substrate is incubated with HDAC1. 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..

General Information

Materials Supplied

List of component

Item	Amount
HDAC Assay Buffer (10X)	1 vial
HDAC1 (human recombinant)	1 vial
HDAC Trichostatin A	1 vial
HDAC Substrate	1 vial
96-Well Plate (Black)	1 plate
HDAC Developer	2 vials
96-Well Cover Sheet	1 cover

Storage Instruction

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

Item	Storage
HDAC Assay Buffer (10X)	-20℃
HDAC1 (human recombinant)	-80℃
HDAC Trichostatin A	-20℃
HDAC Substrate	-20℃
96-Well Plate (Black)	Room temperature
HDAC Developer	-20℃
96-Well Cover Sheet	Room temperature

Materials Required but Not Supplied

- ✓ A fluorometer capable of measuring fluorescence using excitation wavelengths of 340-360 nm and emission wavelengths of 440-465 nm.
- ✓ Adjustable pipettes and a repeat pipettor
- ✓ A source of UltraPure water (Milli-Q or HPLC-grade water)

Precautions for Use

- Pipetting Hints

- ✓ It is recommended that an adjustable pipette be used to deliver reagents to the wells.
- ✓ 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.

- Precaution
- ✓ The final volume of the assay is 210 µl in all the wells.
- ✓ Use the diluted assay buffer in the assay.
- ✓ All reagents except HDAC1 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 37℃.
- ✓ Monitor the fluorescence with an excitation wavelength of 340-360 nm and an emission wavelength of 440-465 nm

Assay Protocol

Reagent Preparation

- HDAC Assay Buffer (10X) - 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 HDAC1 and for dissolving the HDAC developer. The diluted buffer is stable for six months at 4℃.
- HDAC1 (human recombinant) - The vial contains 125 µl of human recombinant HDAC1. Dilute 100 µl of HDAC1 with 900 µl of diluted assay buffer. The diluted HDAC1 is stable for four hours when stored on ice. If not assaying the entire plate, then adjust the enzyme volume accordingly.
- HDAC Trichostatin A - The vial contains 250 µl of 0.21 mM Trichostatin A. Trichostatin A is an HDAC inhibitor.
- HDAC Substrate - 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 Km value for the HDAC substrate is 100 µM. The final concentration of HDAC substrate in the assay, as described, is 200 µM. This concentration may be reduced by dilution with dimethylsulfoxide at the user's discretion, particularly when assaying for competitive inhibitors.
- HDAC Developer - 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 to the reconstituted developer. One vial of developer will develop the entire plate. The reconstituted developer is stable for two hours.

- Plate Set Up

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. A typical layout of samples and inhibitors to be measured in triplicate is given in Figure 1.

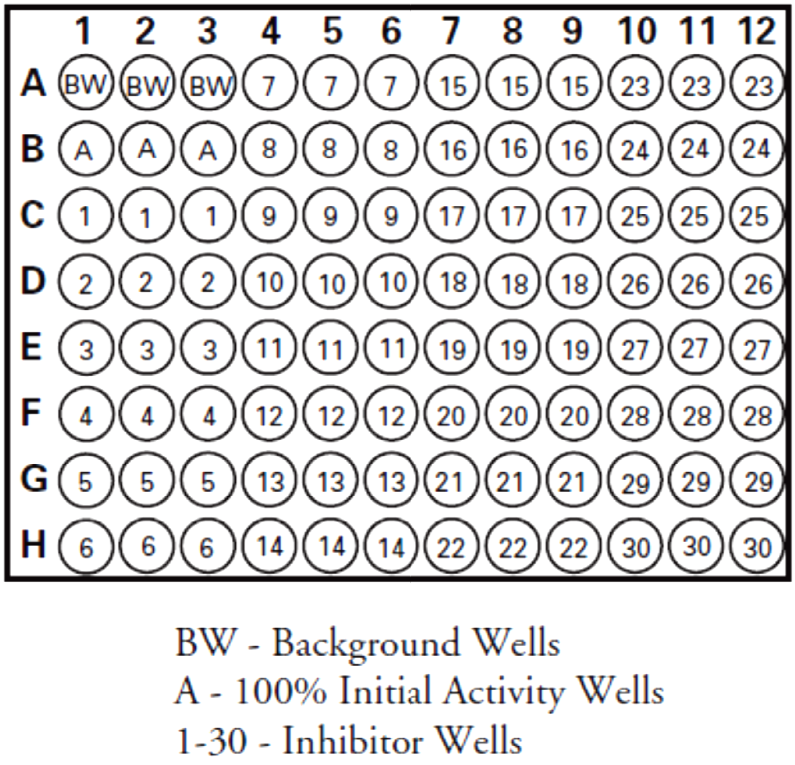

Figure 1. Sample plate format

Assay Procedure

1. 100% Initial Activity Wells - add 140 µl of assay buffer, 10 µl of diluted HDAC1, 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 HDAC1, 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 37℃.
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.

   Note: Inhibitors can be dissolved in assay buffer, ethanol, 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.

Data Analysis

Calculation of Results

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 HDAC1 inhibition by Trichostatin A is shown in Figure 2.

$$\% \text{ Inhibition} = \left[\frac{\text{Initial Activity - Sample}}{\text{Initial Activity}} \right] \times 100$$

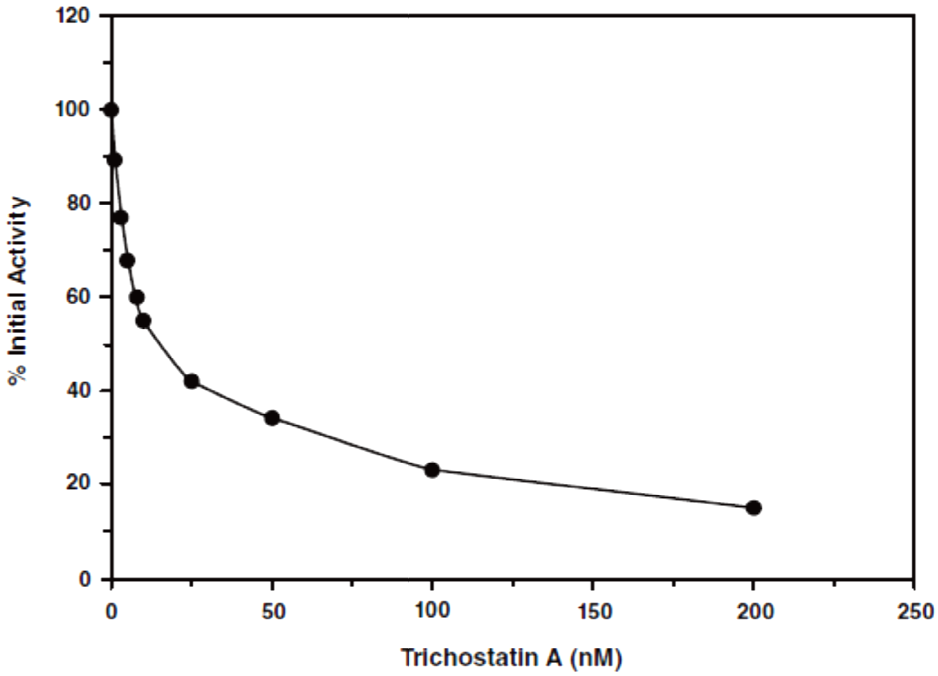

Figure 2. Inhibition of HDAC1 by Trichostatin A (IC_{50} = 12 nM; final concentration of HDAC substrate is 200 µM).

Performance Characteristics

- Precision

When a series of eight HDAC1 measurements were performed on the same day, the intraassay coefficient of variation was 2.8%. When a series of eight HDAC1 measurements were performed on five different days under the same experimental conditions, the inter-assay coefficient of variation was 2.7%.

Resources

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Carefully tap the side of the plate with your finger to remove bubbles B. Be careful not to splash the contents of the wells
No fluorescence above background is seen in the Inhibitor wells	Inhibitor concentration is too high and inhibited all of the enzyme activity	Reduce the concentration of the inhibitor and re-assay
Fluorescence value was at the maximal level in the sample Wells	A. The sample is too concentrated B. The Gain setting is set too high	Make sure you diluted the HDAC1 correctly. Set the gain to a lower setting and measure the fluorescence
No inhibition was seen with the inhibitor	A. The inhibitor concentration is not high enough B. The compound is not an inhibitor of the enzyme	Increase the inhibitor concentration and re-assay

Reference

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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).