HDAC1 Inhibitor Screening Assay Kit

Item No. 10011564

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Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -80°C kit. After opening the kit, store the individual components as stated below.

Item Number	Item	Quantity	Storage
10006389	HDAC Assay Buffer (10X)	1 vial	-20°C
10011618	HDAC1 (human recombinant)	1 vial	-80°C
10006391	HDAC Trichostatin A	2 vials	-20°C
10006392	HDAC Substrate	1 vial	-20°C
400017	96-Well Plate (Black)	1 plate	RT
10006394	HDAC Developer	2 vials	-20°C
400012	96-Well Cover Sheet	1 cover	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

Sold under license from CycLex® Co., Ltd (Patent Nos. EP 1243658, US 7033778, US 7256013, and JP 4267043).

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

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 Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 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 as directed in the **Materials Supplied** section, on page 3, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A fluorometer capable of measuring fluorescence using excitation wavelengths of 340-360 nm and emission wavelengths of 440-465 nm.
2. Adjustable pipettes and a multichannel or repeating pipette.
3. A source of UltraPure water (Milli-Q or HPLC-grade water).

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]

About This Assay

Cayman's 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.

PRE-ASSAY PREPARATION

Reagent Preparation

1. **HDAC Assay Buffer (10X) - (Item No. 10006389)**

   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°C.

2. **HDAC1 (human recombinant) - (Item No. 10011618)**

   The vial contains 125 µl of partially purified human recombinant HDAC1. Dilute 100 µl of HDAC1 with 900 µl of diluted Assay Buffer. Some turbidity is expected. The diluted HDAC1 is stable for four hours when stored on ice. If not assaying the entire plate, then adjust the enzyme volume accordingly.

3. **HDAC Trichostatin A - (Item No. 10006391)**

   The vial contains 250 µl of 0.21 mM Trichostatin A in dimethylsulfoxide (DMSO). Add 100 µl of the Trichostatin A to the Developer Solution (Step 5). The remaining Trichostatin A is used as a positive control inhibitor. The addition of 10 µl to the assay yields a final concentration of 12.35 µM Trichostatin A in the well.

4. **HDAC Substrate - (Item No. 10006392)**

   The vial contains 1.2 ml of 3.4 mM acetylated fluorometric substrate in DMSO. The solution is ready to use as supplied. *NOTE: The K_m 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 DMSO at the user's discretion, particularly when assaying for competitive inhibitors.*

5. **HDAC Developer - (Item No. 10006394)**

   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 (Item No. 10006391) to the reconstituted Developer. One vial of Developer will develop the entire plate. The reconstituted Developer is stable for two hours.

ASSAY PROTOCOL

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 on the template sheet provided (see page 14). A typical layout of samples and inhibitors to be measured in triplicate is given in Figure 1.

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

BW - Background Wells
A - 100% Initial Activity Wells
P - Positive Control Wells
1-29 - Inhibitor Wells

Figure 1. Sample plate format

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.

General Information

- 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.
- Twenty-nine inhibitor samples can be assayed in triplicate or forty-five in duplicate.
- The assay temperature is 37°C.
- Monitor the fluorescence with an excitation wavelength of 340-360 nm and an emission wavelength of 440-465 nm.

Performing the Assay

1. **Background Wells** - add 150 µl of Assay Buffer and 10 µl of solvent (the same solvent used to dissolve the inhibitor) to three wells.
2. **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.
3. **Positive Control Inhibitor Wells** - add 140 µl of Assay Buffer, 10 µl of diluted HDAC1, and 10 µl of HDAC Trichostatin A to three wells. The final concentration of the Trichostatin A in the positive control inhibitor wells is 12.35 µM.
4. **Inhibitor Wells** - add 140 µl of Assay Buffer, 10 µl of diluted HDAC1, and 10 µl of inhibitor* to three wells.
5. Initiate the reactions by adding 10 µl of HDAC Substrate to all the wells being used.
6. Cover the plate with the plate cover and incubate on a shaker for 30 minutes at 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 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, ethanol, methanol, or DMSO 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.

ANALYSIS

Calculations

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, on page 12.

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

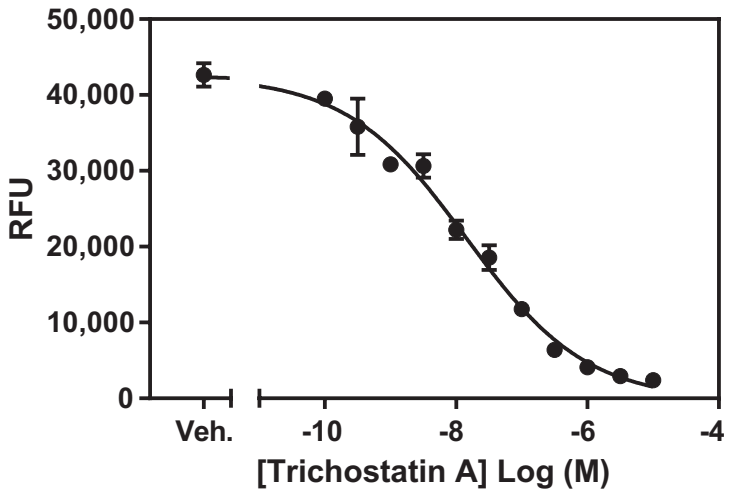

Figure 2. Inhibition of HDAC1 by Trichostatin A. Shown is a typical inhibition curve using this kit. "Veh." represents compound vehicle control.

Performance Characteristics

Precision:

When a series of eight HDAC1 measurements were performed on the same day, the intra-assay 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. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
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

References

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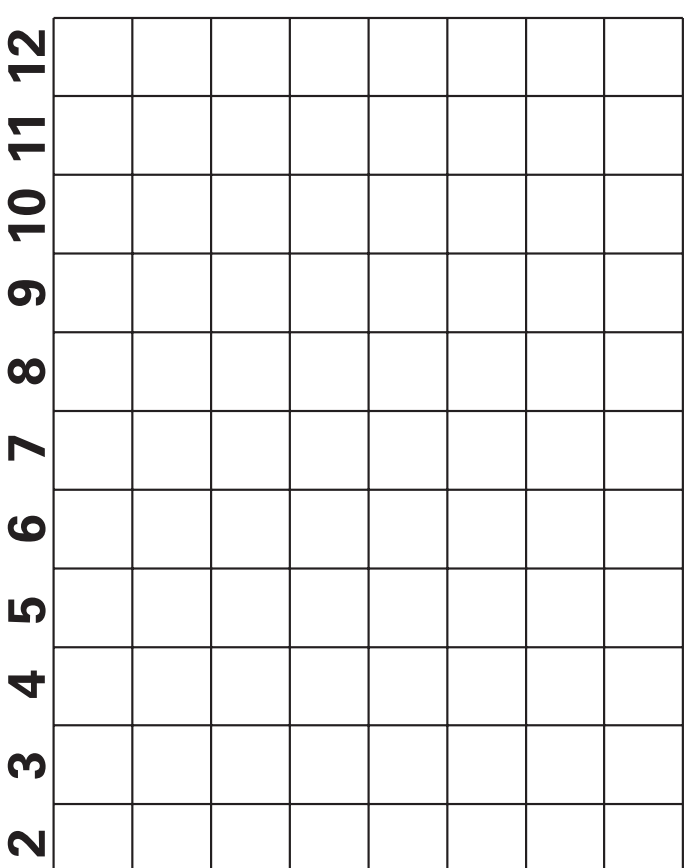
4. Kwon, H.J., Kim, M.S., Kim, M.J., *et al. Int. J. Cancer* **97**, 290-296 (2002).
5. Suenaga, M., Soda, H., Oka, M., *et al. Int. J. Cancer* **97**, 621-625 (2002).
6. Johnstone, R.W. *Nature Reviews Drug Discover* **1**, 287-299 (2002).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.