HDAC1 Inhibitor Screening Assay Kit

Item No. 10011564

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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) Assay Reagent	2 vials	-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.

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

Email: techserv@caymanchem.com

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, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*

INTRODUCTION

Background

Histone deacetylase 1 (HDAC1) is a class I HDAC that catalyzes the deacetylation of histones and non-histone proteins, including transcription factors and various enzymes.[1] It decreases lysine acetylation of histone tails, preventing transcriptional machinery and transcription factors from accessing gene promotors thereby suppressing transcription.[1,2] HDAC1 also regulates both acute and chronic adaptation to environmental stimuli through its regulation of enzyme activity via deacetylation and has roles in various processes including erythropoiesis, senescence, adult neurogenesis, redox homeostasis, angiogenesis, and aging.[1-3] HDAC1 has diverse roles in the pathology of disease, and pharmacologic inhibition of HDAC1 has shown promise in the modulation of cancer, neurodegeneration, inflammatory disorders, and cardiovascular disease.[4]

About This Assay

Cayman's HDAC1 Inhibitor Screening Assay Kit provides a fast, fluorescence-based method for screening HDAC1 inhibitors. The procedure consists of 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. Trichostatin A is included as a control inhibitor. The fluorophore can be easily analyzed using a fluorescence plate reader with an excitation wavelength of 340-360 nm and an emission wavelength of 440-465 nm.

Reagent Preparation

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

   Dilute 5 ml of HDAC Assay Buffer (10X) with 45 ml of ultrapure water. The diluted buffer is stable for six months at 4°C.

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

   Each vial contains 60 µl of human recombinant HDAC1. Dilute 50 µl of HDAC1 with 450 µl of diluted assay buffer. This is a sufficient volume to assay 50 wells. Some turbidity is expected. The diluted HDAC1 is stable for four hours when stored on ice. If not using the undiluted enzyme all at once, prepare aliquots and store at -80°C. Avoid multiple freeze-thaw cycles.

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

   The vial contains 250 µl of 0.21 mM Trichostatin A in DMSO. It is ready to use as supplied. Trichostatin A is an HDAC inhibitor which is used both as a control and in preparing the developer solution.

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 at the user's discretion by dilution with DMSO. Using a lower concentration of substrate may be advantageous when assaying for competitive inhibitors.*

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

   Reconstitute one vial of HDAC Developer with 4 ml of diluted assay buffer and add 100 µl of HDAC Trichostatin A (Item No. 10006391). Store the prepared developer on ice where it will be stable for two hours. One vial of HDAC Developer provides a sufficient volume to assay 100 wells.

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 vehicle control and three wells designated as background wells. It is suggested that each test compound be assayed in triplicate and that the contents of each well are recorded on the template sheet provided (see page 14). A suggested plate format is shown in Figure 1 below.

	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	V	V	V	7	7	7	15	15	15	23	23	23
C	C	C	C	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
V = Vehicle Control Wells
C = Control Inhibitor Wells
1-29 = Test Wells

Figure 1. Sample plate format

Pipetting Hints

- It is recommended that a multichannel pipette 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., 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 HDAC 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.
- We recommend assaying test compounds in triplicate, but it is the user's discretion to do so.
- Twenty-nine compounds 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.
- Test compounds can be dissolved in assay buffer, ethanol, methanol, or DMSO and should be added to the assay in a final volume of 10 µl. It is recommend to assay several concentrations of the test compounds.

Performing the Assay

1. **Background Wells** - add 150 µl of diluted assay buffer and 10 µl of solvent used to dissolve the test compounds/control inhibitor to three wells. If different solvents are to be assayed at the same time, separate sets of background wells should be run for each solvent.
2. **Vehicle Control Wells** - add 140 µl of diluted assay buffer, 10 µl of diluted HDAC1, and 10 µl of solvent used to dissolve the test compounds/control inhibitor to three wells. If different solvents are to be assayed at the same time, separate sets of vehicle control wells should be run for each solvent.
3. **Control Inhibitor Wells** - add 140 µl of diluted 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 well is 12.35 µM.
4. **Test Wells** - add 140 µl of diluted assay buffer, 10 µl of diluted HDAC1, and 10 µl of test compound 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 prepared 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.

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. Using the corrected values, determine the percent inhibition or percent activity for each test compound using one of the following equations:

$$\% \text{ inhibition} = \left[1 - \frac{\text{corrected value of test compound}}{\text{corrected value of vehicle}}\right] \times 100$$

$$\% \text{ activity} = \frac{\text{corrected value of test compound}}{\text{corrected value of vehicle}} \times 100$$

4. Graph the percent inhibition or percent activity as a function of test compound 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 11.

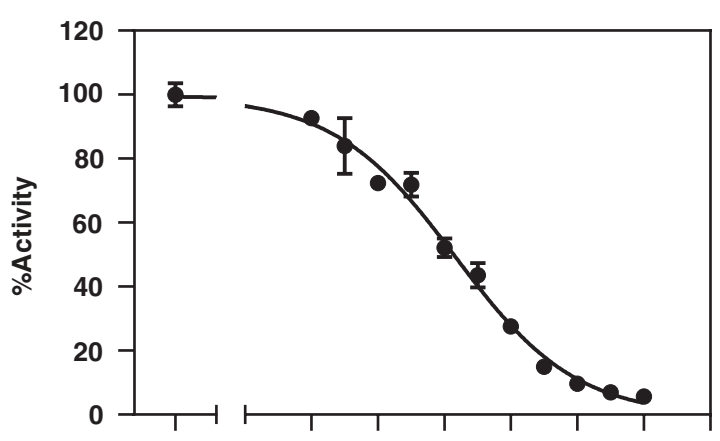


Figure 2. Inhibition of HDAC1 by Trichostatin A. Shown is a typical inhibition curve using this kit. "Veh." represents compound vehicle control. The IC_{50} value of trischostatin A in this experiment is 15 nM.

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%.

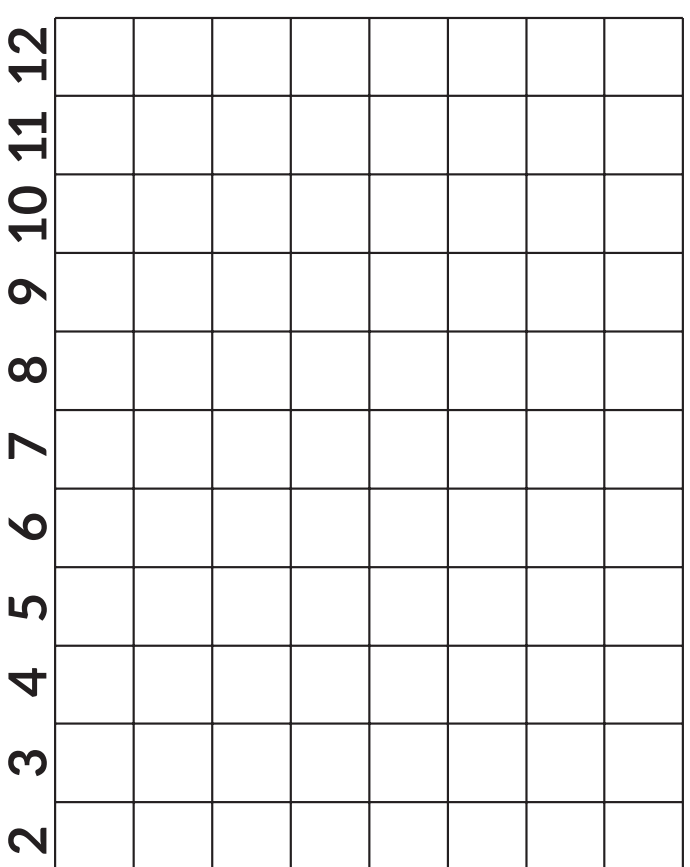
RESOURCES

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 wells	A. The enzyme or substrate was not added to the well(s) or the enzyme has degraded B. All of the enzyme activity is inhibited	A. Make sure to add all components to the wells. Use the reagents immediately or store on ice for not more than two hours B. Reduce the concentration of the inhibitor and test again
% Activity in test compound/inhibitor control wells are comparable to the vehicle control	A. The enzyme is too concentrated B. The gain setting is set too high C. The concentration is not high enough D. The test compound is not an inhibitor	A. Make sure you diluted the HDAC1 correctly B. Set the gain to a lower setting and measure the fluorescence C. Increase the concentration and re-assay to verify

References

1. Dunaway, L.S. and Pollock, J.S. HDAC1: An environmental sensor regulating endothelial function. *Cardiovasc. Res.* **118(8)**, 1885-1903 (2022).
2. Kim, M.Y., Yan, B., Huang, S., *et al.* Regulating the regulators: The role of histone deacetylase 1 (HDAC1) in erythropoiesis. *Int. J. Mol. Sci.* **21(22)**, 8640 (2020).
3. Willis-Martinez, D., Richards, H.W., Timchenko, N.A., *et al.* Role of HDAC1 in senescence, aging, and cancer. *Exp. Gerontol.* **45(4)**, 279-285 (2010).
4. Yoon, S. and Eom, G.H. HDAC and HDAC inhibitor: From cancer to cardiovascular diseases. *Chonnam. Med. J.* **52(1)**, 1-11 (2016).



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.