HDAC Cell-Based Activity Assay Kit

Item No. 600150

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TABLE OF CONTENTS

GENERAL INFORMATION
3 Materials Supplied
4 Safety Data
4 Precautions
4 If You Have Problems
5 Storage and Stability
5 Materials Needed but Not Supplied

INTRODUCTION
6 Background
6 About This Assay

PRE-ASSAY PREPARATION
7 Reagent Preparation

ASSAY PROTOCOL
9 Plate Set Up
11 Standard Curve Preparation
12 Performing the Assay

ANALYSIS
14 Calculations
16 Performance Characteristics

RESOURCES
17 Troubleshooting
17 References
18 Plate Template
19 Notes
19 Warranty and Limitation of Remedy

GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -80°C kit. For best results, remove components and store as stated below.

Item Number	Item	Quantity/Size	Storage
10006389	HDAC Assay Buffer (10X)	1 vial/5 ml	-20°C
600009	Nonidet P-40 Assay Reagent (10%)	1 vial/500 µl	RT
10006393	HDAC Deacetylated Standard	1 vial/400 µl	-20°C
10011618	HDAC1 (human, recombinant) Assay Reagent	1 vial/50 µl	-80°C
600155	Cell-Based Assay HDAC Substrate	1 vial/100 µl	-20°C
10006394	HDAC Developer	1 vial/20 mg	-20°C
10006391	HDAC Trichostatin A	1 vial/250 µl	-20°C
400092	96-Well Clear Bottom Black Culture Plate	1 plate	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

The kit will perform as specified if components are stored as directed on page 3 and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Materials needed for cell culture
2. A fluorometer with the capacity to measure fluorescence using an excitation wavelength of 340-360 nm and an emission wavelength of 440-465 nm
3. Adjustable pipettes and a repeating pipettor
4. 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 deacetylases (HDACs), also known as lysine deacetylases, are a family of enzymes that catalyze the deacetylation of proteins.[1,2] The HDAC enzyme family is grouped into four classes: Class I Rpd3-like enzymes (HDAC1, -2, -3, and -8), Class II Hda1-like enzymes (HDAC4-7, -9, and -10), Class III Sir2-like enzymes, which contains sirtuins that function as protein deacetylases and/or ADP ribosylases, and the Class IV enzyme HDAC11.[3] Class I and Class II HDACs are zinc-dependent enzymes and Class I HDACs are NAD-dependent enzymes.[4] HDACs are nuclear transcriptional regulatory proteins that regulate transcription factor activity and chromosome structure by removing acetyl groups from histones.[1,4] Additionally, they facilitate post-translational modification of non-histone proteins, such as NF-κB, heat-shock proteins (HSPs), and MAPK, to regulate diverse biological pathways. HDACs are implicated in several diseases, including cancer, pulmonary fibrosis, rheumatoid arthritis, and neurodegenerative disease.[4,5]

About This Assay

Cayman's HDAC Cell-Based Assay Kit provides a robust and easy-to-use platform for studying HDAC activity modulators in whole cells. By using a cell-permeable HDAC substrate, the activity of various lysine-specific protein deacetylases can be measured in intact cells in a simple and homogenous manner. The fluorescence of the deacetylated reaction product can be analyzed using a fluorometer with an excitation wavelength of 340-360 nm and an emission wavelength of 440-460 nm. An HDAC inhibitor, trichostatin A, is included for checking specificity of the HDAC reaction. This assay parallels Cayman's HDAC Activity Assay Kit (Item No. 10011563), which uses a nuclear extract rather than whole cells for the assay. Together, both assays will help identify potential inhibitors/activators of HDAC enzymes.

PRE-ASSAY PREPARATION

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 assay buffer is stable for six months at -20°C.

2. **HDAC Deacetylated Standard - (Item No. 10006393)**

   The vial contains 400 µl of 2.1 mM deacetylated standard in DMSO, which is ready to use as supplied. It will be stable for 12 months when stored at -20°C. Avoid multiple freeze-thaw cycles.

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

   This vial contains 50 µl of human recombinant HDAC1, which is used as a positive control. Dilute 10 µl of the enzyme with 190 µl of diluted assay buffer. 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.

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

   This vial contains 250 µl of 0.21 mM trichostatin A (TSA). TSA is an HDAC inhibitor which is used both as a control and in preparing the developer solution. Dilute 50 µl of the TSA stock with 450 µl of diluted assay buffer. A 10 µl aliquot in the assay results in a final concentration of 2.1 µM.

5. **Cell-Based Assay HDAC Substrate - (Item No. 600155)**

   The 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.

6. **Nonidet P-40 Assay Reagent (10%) - (Item No. 600009)**

   This vial contains 500 µl of 10% nonidet P-40 detergent, which is ready to use to prepare the lysis/developer mixture.

7. **HDAC Lysis/Developer Mixture**

   NOTE: Prepare the Lysis/Developer Mixture immediately before use in the assay.

   To one vial of HDAC Developer (Item No. 10006394), add 4.5 ml of diluted assay buffer, 72 µl of undiluted HDAC Trichostatin A, and 500 µl of Nonidet P-40 Assay Reagent. The addition of TSA in the lysis/developer mixture quenches the reaction.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical layout of standards and samples to be measured in duplicate is shown in Figure 1, below. It is suggested that the contents of each well are recorded on the template sheet provided (see page 18).

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1	S5	S5	S9	S9	S13	S13	S17	S17
B	B	B	B1	B1	B5	B5	B9	B9	B13	B13	B17	B17
C	C	C	S2	S2	S6	S6	S10	S10	S14	S14	S18	S18
D	D	D	B2	B2	B6	B6	B10	B10	B14	B14	B18	B18
E	E	E	S3	S3	S7	S7	S11	S11	S15	S15	S19	S19
F	F	F	B3	B3	B7	B7	B11	B11	B15	B15	B19	B19
G	IC	IC	S4	S4	S8	S8	S12	S12	S16	S16	S20	S20
H	PC	PC	B4	B4	B8	B8	B12	B12	B16	B16	B20	B20

A-F = Standard Wells
IC = Inhibitor Control Wells
PC = Positive Control Wells
S1-S20 = Sample Wells
B1-B20 = Background 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.

Standard Curve Preparation

Label six clean test tubes A-F. Pipette 1,400 µl of diluted assay buffer to tube A. Pipette 500 µl of diluted assay buffer to tubes B-F. Transfer 20 µl of the HDAC Deacetylated Standard to tube A. Mix gently. Serially dilute the standard by removing 500 µl from tube A and placing it into tube B. Mix gently. Next, remove 500 µl from tube B and place it into tube C. Mix gently. Repeat the process for tubes D-E. Do not add any standard to tube F. This tube is the zero point of the standard curve. The standards will be stable at room temperature for up to one hour.

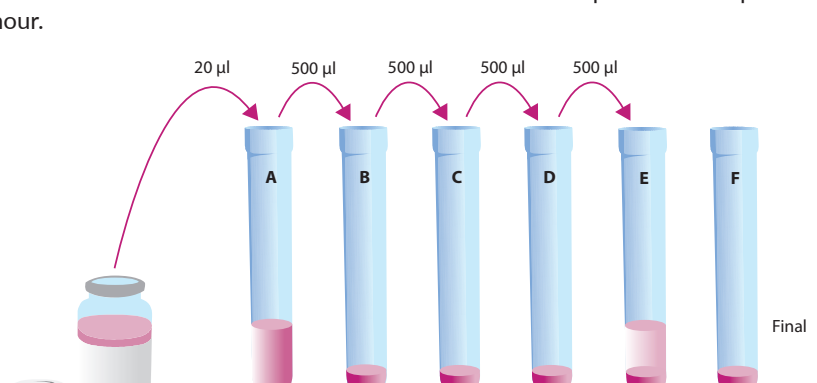

Figure 2. Preparation of standards

Performing the Assay

It is recommended not to exceed 80% confluence by the time of the assay. Optimal conditions will depend on the cell type.

1. Culture cells in the provided plate and treat the cells with experimental compounds or vehicle controls in a CO_2 incubator at 37°C overnight or until the cells have reached 80% confluence. Perform each treatment in quadruplicate where two of the four wells will be used as **Sample Wells** and the other two as B**ackground Wells.**
   a. **Standard Wells:** designate 12 empty wells for the standards (see **Sample Plate Format** on page 9).
   b. **Positive Control Wells:** designate two empty wells for the HDAC1 positive control (see **Sample Plate Format** on page 9).
   c. **Inhibitor Control Wells:** designate two empty wells for the HDAC1 positive control treated with TSA.
2. Centrifuge the plate at 500 x g for 5 minutes before carefully aspirating the culture medium.
3. Add 200 µl of diluted assay buffer to all wells. Centrifuge the plate as in step 2 and carefully aspirate the buffer.
4. Add the appropriate amount of the prepared reagent(s) to the designated wells as follows:

Reagent	Standard (A-F)	Positive Control (PC)	Inhibitor Control (IC)	Sample (S1-S20)	Background (B1-B20)
Culture Medium	--	--	--	80 µl	80 µl
Standards	100 µl	--	--	--	--
Positive Control	--	80 µl	80 µl	--	--
TSA	--	--	10 µl	--	10 µl
Diluted Assay Buffer	--	10 µl	--	10 µl	--

5. Initiate the reaction by adding 10 µl of the diluted HDAC substrate to all wells except the standard wells.
6. Incubate the plate at 37°C in a CO_2 incubator for 1-3 hours. The optimal incubation time will depend on cell type.
7. Add 50 µl of the prepared lysis/developer mixture (see **Reagent Preparation** on page 7) to all wells and shake the plate for 1 minute before incubating for 15 minutes at 37°C.
8. Read fluorescence using an excitation wavelength of 340-360 nm and an emission wavelength of 440-460 nm.

ANALYSIS

Calculations

Plot the Standard Curve

1. Determine the average fluorescence of the standards. Subtract the fluorescence value of the zero standard (Standard F) from itself and all other standards. This is the corrected fluorescence value.
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 3, on page 15, for a typical standard curve.

Determination of HDAC activity

1. For each treatment, determine the average fluorescence of both sample and background wells.
2. Subtract the background fluorescence from the corresponding 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.

$$\text{HDAC Activity (nmol/min/ml)} = \frac{\text{Corrected Fluorescence Value - (y-intercept)}}{\text{Slope x Incubation Time (60-180 minutes)}}$$

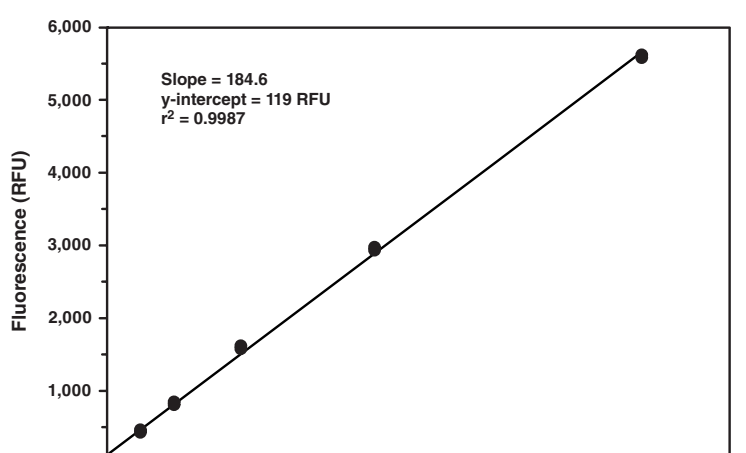

Figure 3. Typical standard curve

Performance Characteristics

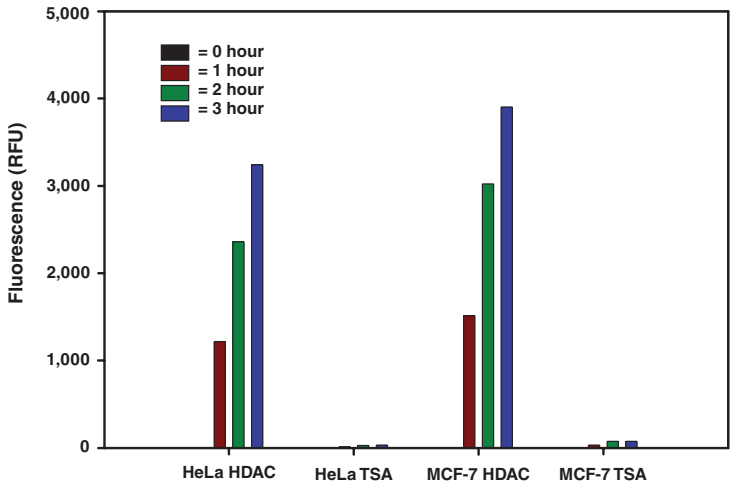

Figure 4. HDAC activity in HeLa cells and MCF-7 cells. HeLa cells and MCF-7 cells were seeded in a 96-well clear bottom black plate at a density of 4 x 10^4 cells/well. The next day, cells were processed for the measurement of HDAC activity according to the protocol described in this booklet.

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	Reduce and optimize cell seeding density

References

1. Dunaway, L.S. and Pollock, J.S. HDAC1: An environmental sensor regulating endothelial function. *Cardiovasc. Res.* **118(8)**, 1885-1903 (2022).
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3. Li, Y. and Seto, E. HDACs and HDAC inhibitors in cancer development and therapy. *Cold Spring Harb. Perspect. Med.* **6**, a026831 (2016).
4. Zhang, H., Ji, L, Yang, Y., *et al.* The rolde of HDACs and HDACi in cartilage and osteoarthritis. *Front. Cell Dev. Biol.* **8**, 560117 (2020).
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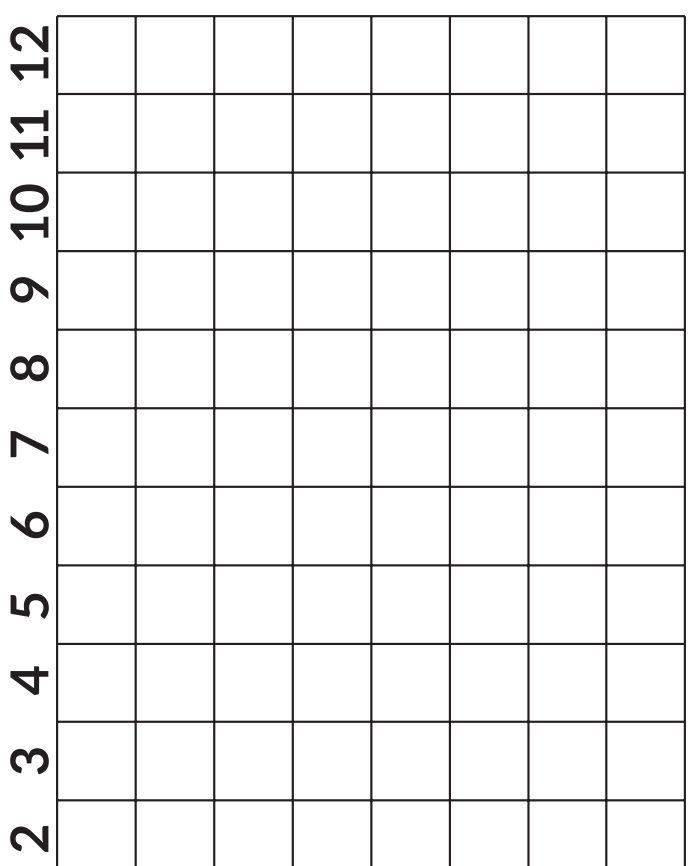

NOTES

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.

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