HDAC Fluorometric Activity Assay Kit

Item No. 10011563

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Technical Support 888.526.5351
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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	Storage
10006389	HDAC Assay Buffer (10X)	1 vial	-20°C
10011618	HDAC1 (human, recombinant) Assay Reagent	1 vial	-80°C
10006391	HDAC Trichostatin A	1 vial	-20°C
10006392	HDAC Substrate	1 vial	-20°C
10006393	HDAC Deacetylated Standard	1 vial	-20°C
10006394	HDAC Developer	2 vials	-20°C
400017	96-Well Plate (Black)	1 plate	RT
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 at -80°C 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 repeating pipettor.
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).*
4. Materials needed for sample preparation

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 Fluorometric Activity Assay Kit provides a fast, fluorometric method for measuring HDAC activity without the use of radioactivity, extraction, or chromatography. In the first step, an acetylated lysine substrate is incubated with samples. 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. The assay can be used for quantifying Class I and II HDAC activity from various sources.

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 buffer is stable for six months at 4˚C.

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

   The vial contains 50 µl of human recombinant HDAC1, which can be used as a positive control. Dilute 10 µl of HDAC1 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.

3. **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.

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.*

5. **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.

6. **HDAC Developer Mixture**

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

   To one vial of HDAC Developer (Item No. 10006394), add 4 ml of assay buffer and 100 µl of undiluted HDAC Trichostatin A. The addition of TSA in the developer mixture quenches the reaction.

Sample Preparation

Nuclear Extracts

It is recommended that Cayman's Nuclear Extraction Kit (Item No. 10009277) be used to isolate and extract nuclear contents from cell culture and tissues. If not assaying immediately, samples should be frozen and stored at -80°C. A minimum of 100 µl of sample is required to have a sufficient volume to run the assay in duplicate. To fall within the range of the assay, it may be necessary to dilute samples several times with the diluted assay buffer prior to the assay.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. It is suggested that each sample and standard be assayed at least in duplicate (triplicate is preferred). A typical layout of standards and samples to be measured in duplicate given below in Figure 1. It is suggested that the contents of each well are recorded on the template sheet provided (see page 21).

	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 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 the HDAC1 enzyme, samples, 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, however, a standard curve should be run each time.
- It is recommended that the samples and standards be assayed at least in duplicate (triplicate recommended).
- Twelve samples in triplicate or twenty samples in duplicate can be run in the assay.
- 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.

Standard Preparation

Label six clean test tubes A-F. Pipette 910 µl of diluted assay buffer to tube A. Pipette 400 µl of diluted assay buffer to tubes B-F. Transfer 75 µl of the HDAC Deacetylated Standard to tube A. Mix gently. Serially dilute the standard by removing 400 µl from tube A and placing it into tube B. Mix gently. Next, remove 400 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.

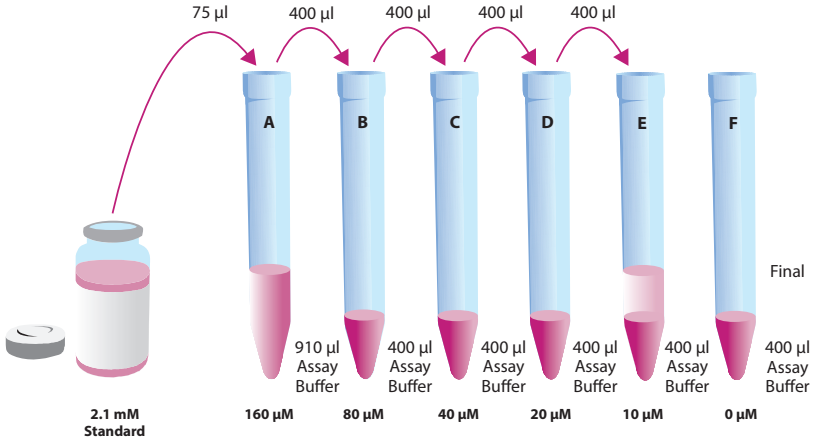


Figure 2. Preparation of standards

Performing the Assay

1. Add the appropriate amount of the prepared reagent(s) to the designated wells according to the table below:

Reagent	Standard	Positive Control (PC)	Inhibitor Control (IC)	Sample (S1-S20)	Background (B1-B20)
Sample	--	--	--	10 µl	10 µl
Standards	10 µl	--	--	--	--
Diluted HDAC1	--	10 µl	10 µl	--	--
Diluted TSA	--	--	10 µl	--	10 µl
Diluted Assay Buffer	150 µl	150 µl	140 µl	150 µl	140 µl

2. Initiate the reactions by adding 10 µl of HDAC Substrate to all the wells.
3. Cover the plate with the 96-Well Cover Sheet (Item No. 400012) and shake for 30 minutes at 37°C.
4. Remove the cover and add 40 µl of prepared HDAC developer mixture (see **Reagent Preparation** on page 9). Cover the plate and incubate for 15 minutes at room temperature.
5. Read fluorescence using an excitation wavelength of 340-360 nm and an emission wavelength of 440-465 nm. The development is stable for 30 minutes.

ANALYSIS

Calculations

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 of the standards (from step 1 above) as a function of the final concentration of deacetylated standard from Figure 2, on page 13. See Figure 3, below, for a typical standard curve.

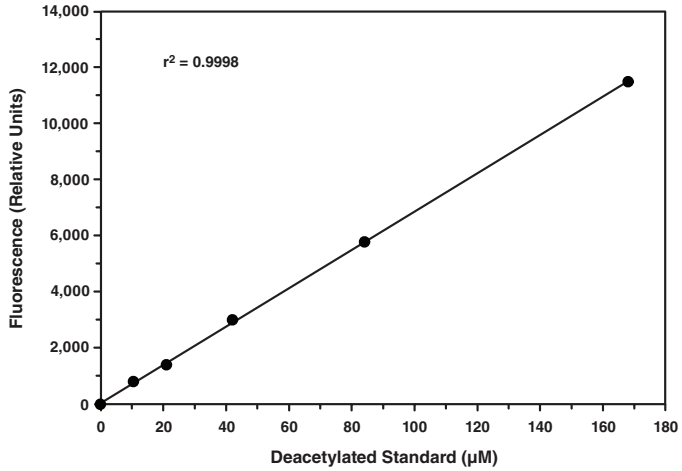


Figure 3. Typical standard curve

3. Determine the average fluorescence values of each sample and background wells.
4. Subtract the background fluorescence from the corresponding sample fluorescence to yield the corrected sample fluorescence values.
5. Calculate the HDAC activity using the equation obtained from the linear regression of the standard curve, substituting corrected fluorescence values for each sample.

$$\text{HDAC Activity (nmol/min/ml)} = \frac{\text{Corrected Fluorescence Value - (y-intercept)}}{\text{Slope x Incubation Time (30 minutes)}}$$

Performance Characteristics

Precision:

When a series of eight HDAC1 measurements were performed on the same day, the intra-assay coefficient of variation was 2.2%. 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 2.4%.

Assay Range:

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.

RESOURCES

Interferences

The following reagents were tested or interference in the assay:

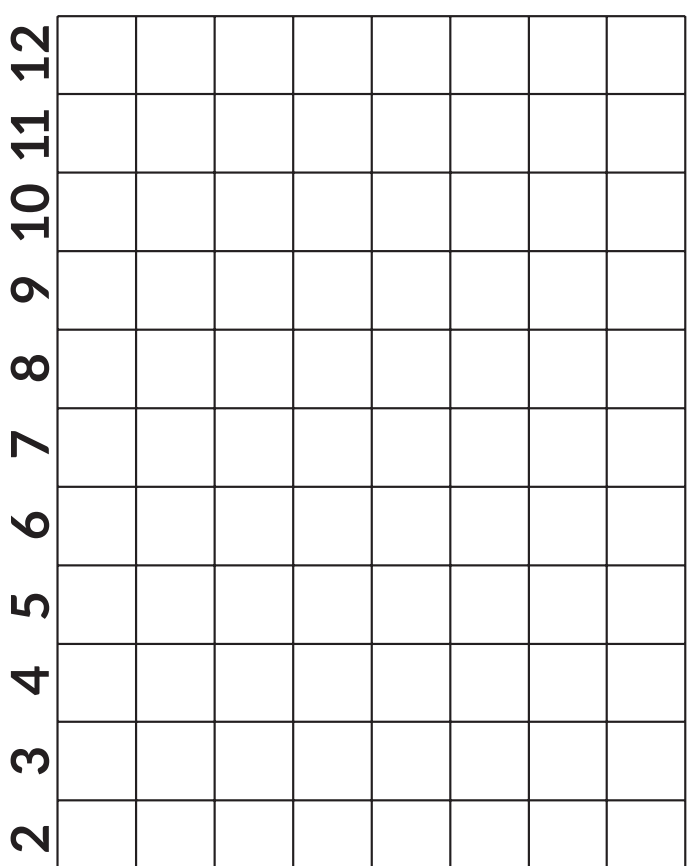
	Reagent	Will Interfere (Yes or No)
Buffers	Tris	No
	Borate	No
	HEPES	No
	Phosphate	No
Protease Inhibitors/ Chelators	≤200 µM PMSF	No
	≤10 µg/ml Leupeptin	No
	≤10 µg/ml Pepstatin	No
	≤10 µg/ml Chymostatin	No
	≤1 mM EGTA	No
	≤1 mM EDTA	No
Solvents	10 µl Ethanol	No
	10 µl Methanol	No
	10 µl Dimethylsulfoxide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

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
HDAC activity was not detected in the sample	Sample was too dilute	Re-assay the sample using a lower dilution
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	A. Dilute samples and re-assay B. Set the gain to a lower setting and re-measure
The deacetylated standard curve did not work	The deacetylated standards were not diluted properly	Set-up the standards according to Figure 2 and re-assay

References

1. Dunaway, L.S. and Pollock, J.S. HDAC1: An environmental sensor regulating endothelial function. *Cardiovasc. Res.* **118(8)**, 1885-1903 (2022).
2. Brancolini, C., Gagliano, T., and Minisini, M. HDACs and the epigenetic plasticity of cancer cells: Target the complexity. *Pharmacol. Ther.* **238**, 108190 (2022).
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).
5. 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.