Description

The HDAC1 Fluorogenic Assay Kit is designed to measure HDAC1 (histone deacetylase 1) activity for screening and profiling applications. The assay kit comes in a convenient 96-well format, with enough purified recombinant HDAC1 enzyme, fluorogenic substrate, HDAC Developer and assay buffer for 100 enzyme reactions. This kit also contains the inhibitor Trichostatin as a control.

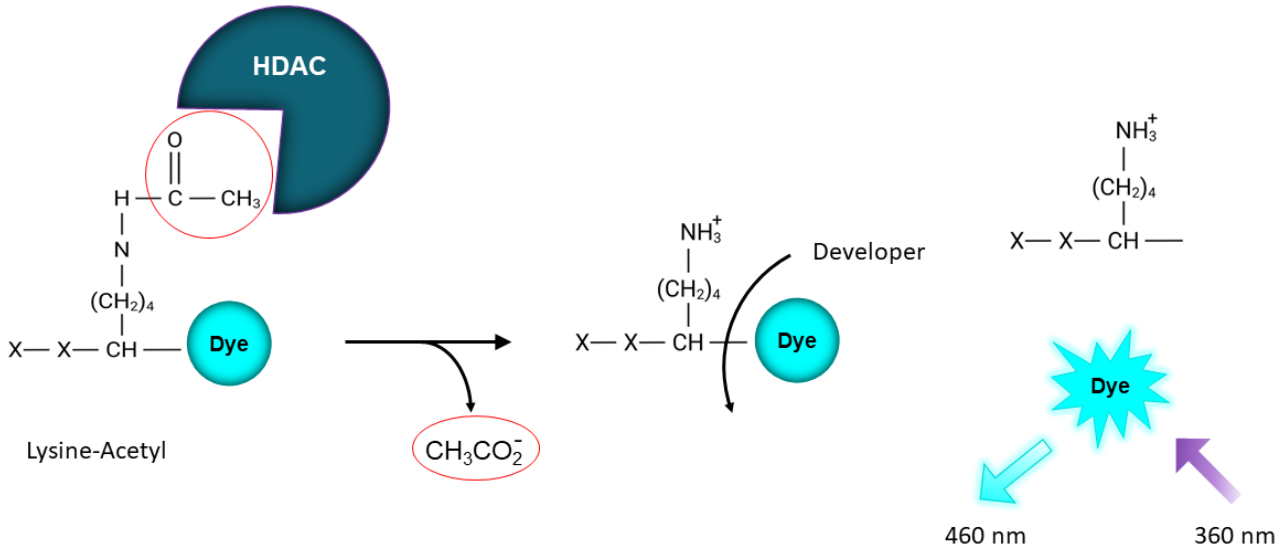

Figure 1: Illustration of the mechanism behind the HDAC1 Fluorogenic Assay Kit.
The fluorescence from dye molecules is quenched when bound to the peptide substrate. HDAC catalyzes the hydrolysis of the acetyl group from the lysine. Upon incubation with a developer solution specific for non-acetylated lysines, the dye is released and able to fluoresce (λex=350-380 nm; λem=440-460 nm). Fluorescence is thus proportional to HDAC activity.

Background

HDAC1, or histone deacetylate 1, is a Class I member of the histone deacetylase family which is involved in lysine deacetylation. Lysine acetylation/deacetylation is a dynamic process involved in the regulation of a variety of cellular functions, similarly to phosphorylation/dephosphorylation. HDAC1 is part of the histone deacetylase complex, localized in the nucleus, and regulates eukaryotic gene expression. It can also directly interact with different transcription factors to regulate specific pathways, for example: HDAC1 interacts with MTA-2 (metastasis-associated protein-2) to deacetylate p53 and thus participates in cell growth and apoptosis regulation. In endothelial cells HDAC regulates angiogenesis, inflammation, redox and nitric oxide signaling, in response to environmental stimuli. Dysfunction of HDAC1 can contribute to atherosclerosis. Mutations in HDAC1 can result in cancer, via the deregulation of genes involved in cell proliferation and survival. Several HDAC inhibitors have been approved for the treatment of cancer, but most are non-selective. Entinostat (or MS-275) is an inhibitor that acts preferentially on HDAC1 and has shown promising results in phase I clinical trials for refractory solid tumors and lymphoma. The development of new inhibitors specifically targeting HDAC1 may open newer avenues for cancer and HDAC1-linked diseases in the endothelium.

Applications

Study enzyme kinetics and screen small molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
50051-KC1	HDAC1, FLAG-Tag, His-Tag*	1 µg	-80°C
50037-KC25	5 mM Fluorogenic HDAC Substrate 3	25 µl	-80°C
50030	2x HDAC Developer (contains 2 µM Trichostatin A)	6 ml	-80°C
82748-KC100	200 µM Trichostatin A	100 µl	-20°C
50031-KC10	HDAC Assay Buffer	10 ml	-20°C
79685	Black, low binding microtiter plate	1	Room Temperature

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- 1 mg/ml BSA (bovine serum albumin) solution in distilled water
- Fluorimeter capable of excitation at λ=350-380 nm and detection at λ=440-460 nm
- Adjustable micropipettor and sterile tips
- Orbital shaker

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

- The final concentration of DMSO in the assay should not exceed 1%.
- Compounds that are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone is tested to determine any potential interference of the compound on the assay results.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include “Blank”, “Positive Control”, “Control Inhibitor” and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

- We recommend using Trichostatin A as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

1. Thaw **5 mM Fluorogenic HDAC Substrate 3, 200 µM Trichostatin A** and **HDAC Assay Buffer.**

2. Prepare the Inhibitor Control by diluting 200 µM Trichostatin A to 1000X the IC_{50} in 100% DMSO. Then dilute 10-fold in HDAC Assay Buffer (the DMSO amount is now 10%) and corresponds to 100X the IC_{50} value (5 µl/ well). Using Diluent Solution prepare solutions at 1X and 10X the IC_{50} value (5 µl/ well).

3. Dilute **5 mM Fluorogenic HDAC Substrate** 50-fold with HDAC Assay Buffer (5 µl/well will be needed). This makes 100 µM Fluorogenic HDAC Substrate 3.

4. Thaw **HDAC1** on ice. Briefly spin the tube to recover the full content.

5. Dilute HDAC1 to 1.4 ng/µl (5 µl/well) with HDAC Assay Buffer.

6. Prepare a **Master Mix** (35 µl/well): N wells x (30 µl of HDAC Assay Buffer 3 + 5 µl of 1 mg/ml BSA).

7. Add 35 µl of Master Mix to every well.

8. Prepare the **Test Inhibitor** (5 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   8.1 If the Test Inhibitor is water-soluble, prepare 10-fold more concentrated serial dilutions of the inhibitor than the desired final concentrations in HDAC Assay Buffer.

   For the positive and negative controls, use HDAC Assay Buffer (Diluent Solution).

   OR

   8.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration 100-fold higher than the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in HDAC Assay Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Using HDAC Assay Buffer containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

   For positive and negative controls, prepare 10% DMSO in HDAC Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO should not exceed 1%.

9. Add 5 µl of **Test Inhibitor** to each well labeled "Test Inhibitor".

10. Add 5 µl of **Diluent Solution** to the “Positive Control" and “Blank” wells.

11. Add 5 µl of **diluted Trichostatin A** to the “Control Inhibitor” wells.

12. Add 5 µl of **HDAC Assay Buffer** to the wells designated as "Blank".

13. Add 5 µl of **diluted HDAC** to the wells designated “Positive Control”, “Control Inhibitor” and "Test Inhibitor".

14. Preincubate the plate for 30 minutes at Room Temperature (RT) with gentle agitation.

15. Initiate the reaction by adding **diluted Fluorogenic HDAC Substrate 3** (100 µM).

16. Incubate at 37°C for 30 minutes.

Component	**Blank**	**Positive Control**	**Control Inhibitor**	**Test Inhibitor**
Master Mix	35 µl	35 µl	35 µl	35 µl
Test Inhibitor	-	-	-	5 µl
Diluted Trichostatin (20 µM)	-	-	5 µl	-
Diluent Solution	5 µl	5 µl	-	-
HDAC Assay Buffer	5 µl	-	-	-
Diluted HDAC (1.4 ng/µl)	-	5 µl	5 µl	5 µl
Diluted Fluorogenic HDAC Substrate 3 (100 µM)	5 µl	5 µl	5 µl	5 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

17. Add 50 µl of **2x HDAC Developer** to each well.

18. Incubate at RT for 15 minutes.

19. Immediately read in a fluorimeter or a microplate reader capable of excitation at λ=350-380 nm and detection at λ=440-460 nm.

20. The “Blank” value should be subtracted from all other readings.

Example Results

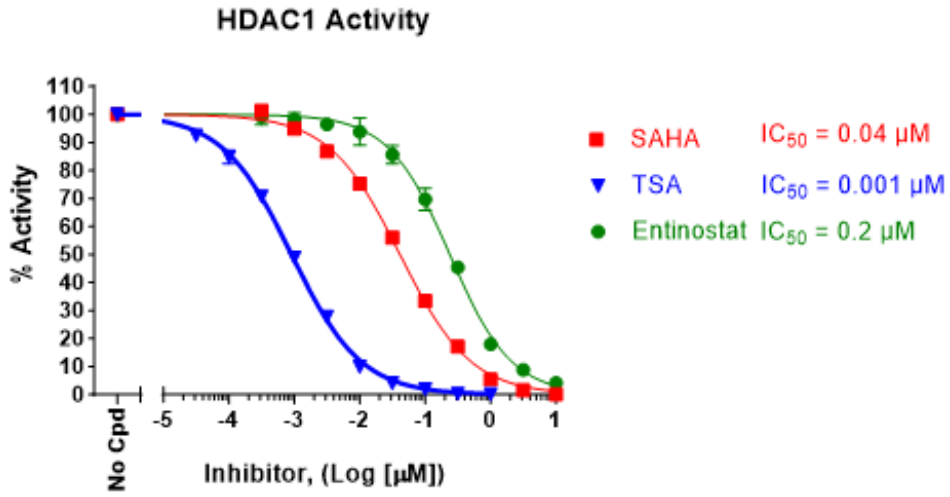

Figure 2: Inhibition of HDAC1 activity by the inhibitors SAHA, TSA (Trichostatin A) and Entinostat. HDAC1 activity was measured in the presence of increasing concentrations of SAHA (Cayman Chemicals #10009929), TSA and Entinostat (EMD Millipore #57625). The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (activity in the absence of inhibitor, set at 100%).

Data shown is representative.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

References

Santo L., *et al.*, 2012 *Blood.* 119(11):2579-89.
Bradner J.E., *et al.*, 2010 *Nat Chem Biol.* 6(3): 238-243.
Dunaway L. and Pollock J., 2022 Cardiovas Res 118(8): 1885-1903.

Related Products

Products	*Catalog #*	*Size*
HDAC1 Kinetic Assay Kit	53001	96 reactions
HDAC1, His-Tag, FLAG-Tag (Mouse) Recombinant	50058	50 µg
Anti-HDAC1 Monoclonal Antibody	25286	50 µg
Anti-HDAC1 Polyclonal Antibody	25287	50 µg
HDAC2 Fluorogenic Assay Kit	50062	96 reactions
HDAC3 Fluorogenic Assay Kit	50073	96 reactions

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