Description

The HDAC4 Fluorogenic Assay Kit is designed to measure HDAC4 (histone deacetylase 4) activity for screening and profiling applications. The assay kit comes in a convenient 96-well format, with enough purified recombinant HDAC4 enzyme (amino acids 627-1084), fluorogenic substrate, HDAC Developer and assay buffer for 100 enzyme reactions. This kit also contains the inhibitor Trichostatin A as a control.

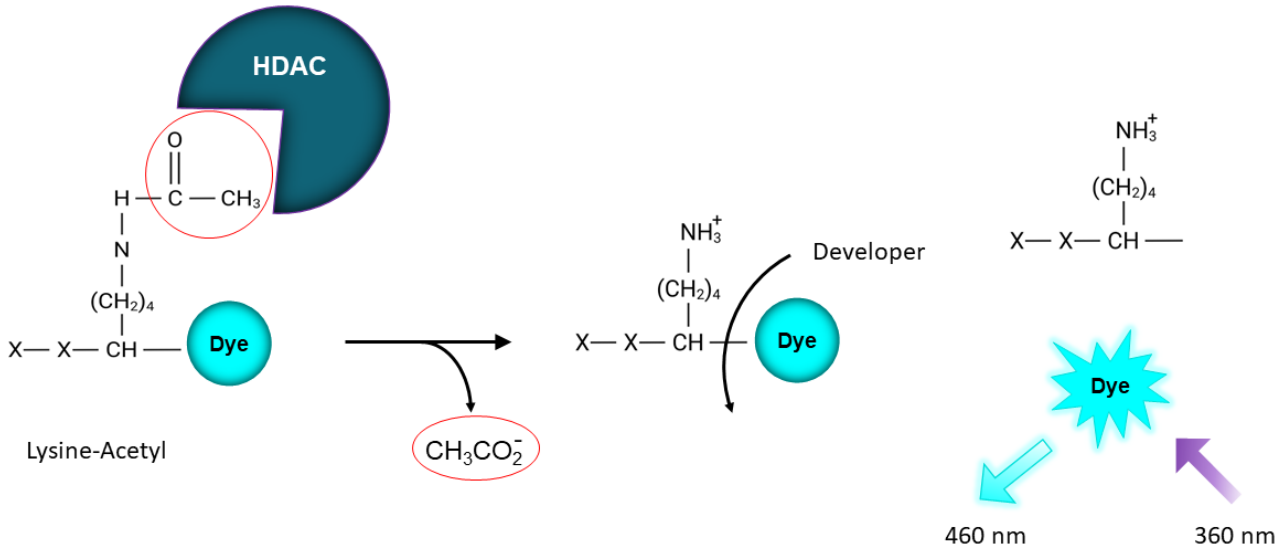

Figure 1: Illustration of the mechanism behind the HDAC4 Fluorogenic Assay Kit.
The fluorescence from dye molecules is quenched when bound to the peptide substrate. HDAC catalyzes the hydrolysis of the acetyl group from the lysine. Upon incubation with a developer solution specific for non-acetylated 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

HDAC4, or histone deacetylate 4, is a Class II 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. It interacts with MEF2C (myocyte-specific enhancer factor 2c) and MEF2D and this interaction allows regulation of gene expression. It also functions in multimeric protein complexes. HDAC4 is regulated by phosphorylation, SUMOylation and proteolytic cleavage, which influence the protein localization in the cell. It is involved in bone and muscle development, and vision. Dysfunction of HDAC4 seems to link to cancer in a context-dependent mode, but the mechanisms behind it are still unclear. It has been linked to multiple myeloma (MM) and acute myeloid leukemia (AML). The development of new inhibitors specifically targeting HDAC4, in a disease-specific context, and a better understanding of its modes of action may open newer avenues of HDAC4-linked diseases.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
50004-KC1	HDAC4, GST-Tag His-Tag*	1 µg	-80°C
50040-KC25	5 mM Fluorogenic HDAC Substrate 2A	25 µl	-80°C
50030	2x HDAC Developer (contains 2 µM Trichostatin A)	6 ml	-80°C
83931-KC100	1 mM 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 with 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.
- Some inhibitors require a pre-incubation step with the target protein before substrate addition to achieve maximum effect or full binding to the target protein. Therefore, depending on the mechanism of action of the tested drugs (e.g., slow-binding inhibitors or covalent inhibitors), it is advisable to include a pre-incubation step in the assay protocol, as longer interaction may reveal time-dependent, potent inhibition missed in short assays, leading to a more accurate determination of the IC_{50} value. Typically, a 30-minute pre-incubation period is sufficient. However, the optimal duration of the pre-incubation phase may vary and must be determined by the end-user.

1. Thaw **5 mM Fluorogenic HDAC Substrate 2A, 1 mM Trichostatin A** and **HDAC Assay Buffer.**

2. Prepare the Inhibitor Control by diluting 1 mM 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 2A** 250-fold with HDAC Assay Buffer (5 µl/well will be needed). This makes 20 µM Fluorogenic HDAC Substrate 2A.

4. Thaw **HDAC4** on ice. Briefly spin the tube to recover the full content.

5. Dilute HDAC4 to 12 pg/µl (5 µl/well) with HDAC Assay Buffer.

6. Prepare a **Master Mix** (40 µl/well): N wells x (30 µl of HDAC Assay Buffer + 5 µl of 20 µM HDAC Substrate 2A + 5 µl of 1 mg/ml BSA).

7. Add 40 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 serial dilutions of the inhibitor 10-fold more concentrated 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 Trichostatin to the “Control Inhibitor” wells.

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

13. Initiate the reaction by adding 5 µl of diluted HDAC4 to the wells designated “Positive Control”, “Control Inhibitor” and "Test Inhibitor".

14. Incubate at 37°C for 30 minutes.

Component	**Blank**	**Positive Control**	**Control Inhibitor**	**Test Inhibitor**
Master Mix	40 µl	40 µl	40 µl	40 µl
Test Inhibitor	-	-	-	5 µl
Diluted Trichostatin	-	-	5 µl	-
Diluent Solution	5 µl	5 µl	-	-
HDAC Assay Buffer	5 µl	-	-	-
Diluted HDAC4 (12 pg/µl)	-	5 µl	5 µl	5 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

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

16. Incubate at RT for 15 minutes.

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

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

Example Results

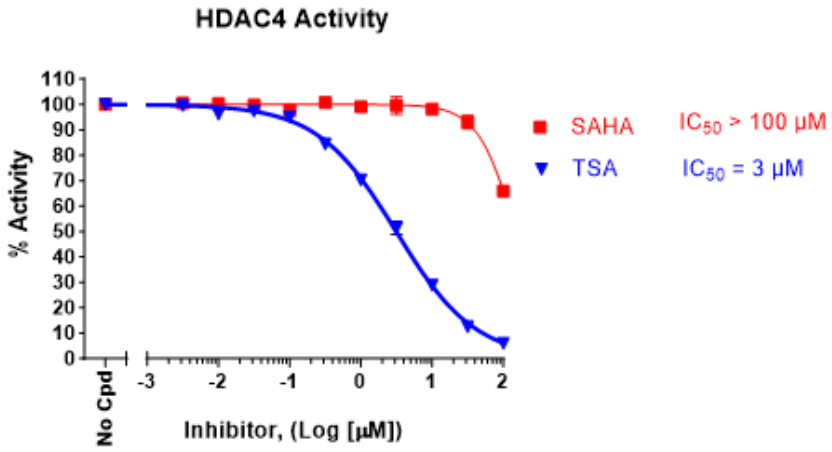

Figure 2: Inhibition of HDAC4 activity by the inhibitors SAHA and TSA (Trichostatin A).
HDAC4 activity was measured in the presence of increasing concentrations of SAHA (Cayman Chemicals #10009929) and TSA following a 30 minute preincubation. The "Blank" value was subtracted from all other values. Results 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
Ontoria. J., *et al.*, 2009 *J. Med. Chem.* 52(21):6782-9.
Cuttini E., *et al.*, 2023 *Front. Mol. Biosci.* 10: 1116660.

Related Products

Products	*Catalog #*	*Size*
HDAC4, GST-Tag Recombinant	100339	10 µg
HDAC1 Fluorogenic Assay Kit	50061	96 reactions
HDAC2 Fluorogenic Assay Kit	50062	96 reactions
HDAC3 Fluorogenic Assay Kit	50073	96 reactions
HDAC1 Kinetic Assay Kit	53001	96 reactions
HDAC2 Kinetic Assay Kit	53002	96 reactions

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