Description

The HDAC Fluorogenic Assay Kit (Green) is designed to measure HDAC (histone deacetylase) class I (HDAC1, 2, and 3) and Class II (HDAC6) activity for screening and profiling applications. The assay kit comes in a convenient 96-well format, with enough fluorogenic substrate, HDAC Developer and assay buffer for 100 enzyme reactions. This kit also contains recombinant purified HDAC2 and the inhibitor Trichostatin as control.

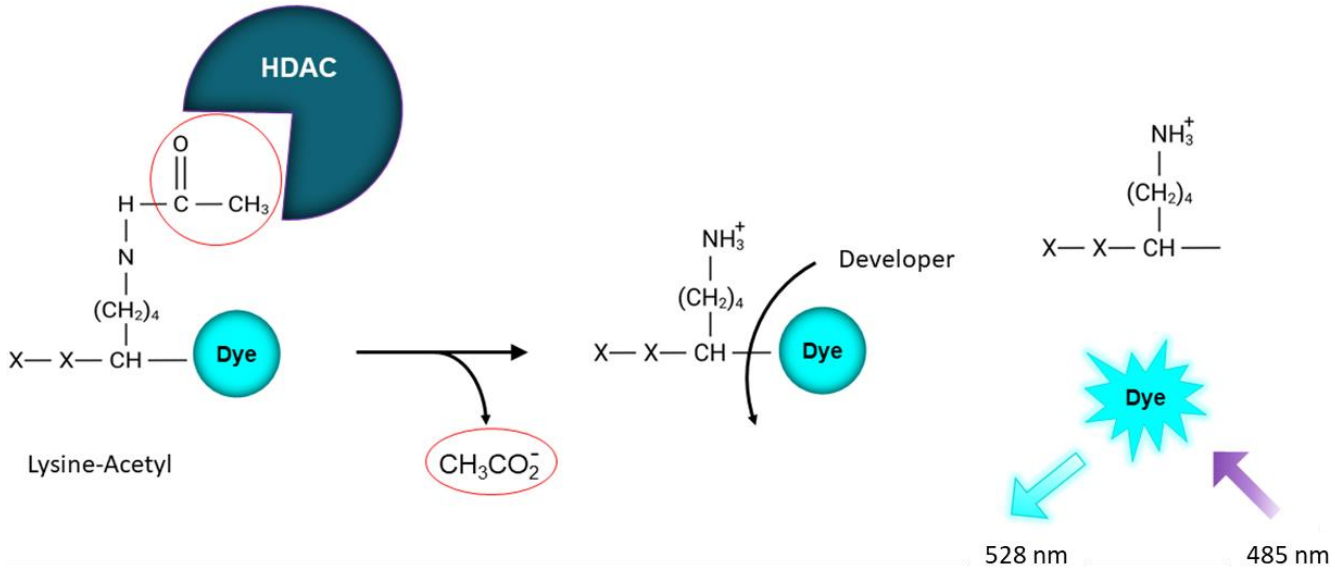

Figure 1: Illustration of the mechanism behind the HDAC Fluorogenic Assay Kit (Green).

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=485 nm; λem=528 nm). Fluorescence is thus proportional to HDAC activity.

Background

HDACs, or histone deacetylases, are a class of proteins involved in lysine deacetylation (removal of acetyl groups). Lysine acetylation/deacetylation is a dynamic process involved in the regulation of a variety of cellular functions, similarly to phosphorylation/dephosphorylation. Acetylation is performed by histone acetyltransferases. There are four classes of HDACs, classified based on sequence homology and domain organization. Class I include HDAC1, 2, 3, and 8, class III includes sirtuin proteins and class IV has only one member, HDAC11. Class II is subdivided into two subclasses, with Class IIA including HDAC4, 5, 7, and 9, and Class IIB including HDAC6 and 10. Mutations in HDACs can lead to pathologies. For instance, dysfunction of 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 HDAC-linked diseases, but most are non-selective. The development of new inhibitors specifically targeting HDAC may open newer avenues for cancer and other HDAC-linked diseases in the endothelium.

Applications

Study enzyme kinetics and screen small molecule inhibitors of HDAC class I (HDAC1, 2, and 3) and HDAC class IIb (HDAC6) that fluoresce in the 440-460 nm wavelength range, for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
50002	HDAC2, His-Tag*	5 µg	-80°C
50038	5 mM Fluorogenic HDAC Substrate 2	25 µl	-80°C
50030	2x HDAC Developer (contains 2 µM Trichostatin A)	6 ml	-80°C
	200 µM Trichostatin A	100 µl	-20°C
50031	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=485 nm and detection at λ=528 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%.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include “Blank”, “Positive Control”, “Control Inhibitor” and “HDAC Test Inhibitor” conditions.
- The amount of HDAC of interest to use may require optimization.
- 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 HDAC Substrate 2, 200 µM Trichostatin A** and **HDAC Assay Buffer.**

2. Prepare the Inhibitor Control by diluting Trichostatin A (200 µM) 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 25-fold the 5 mM HDAC Substrate 2 with HDAC Assay Buffer (5 µl/well will be needed). This makes 200 µM HDAC Substrate 2.

4. Thaw HDAC2 and HDAC of Interest on ice. Briefly spin the tube to recover the full content.

5. Dilute HDAC2 to 6 ng/µl (5 µl/well) with HDAC Assay Buffer.

6. Dilute HDAC of Interest to the optimized concentration (5 µl/well) with HDAC Assay Buffer.

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

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

9. 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.

   9.1 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

   9.2 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%.

10. Add 5 µl of Test Inhibitor to each well labeled “HDAC Test Inhibitor”.

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

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

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

14. Add 5 µl of diluted HDAC2 or diluted HDAC of Interest to the wells designated “Positive Control,” “Control Inhibitor,” and “HDAC Test Inhibitor”.

15. Incubate at 37°C for 30 minutes.

16. Initiate the reaction by 5 µl adding diluted HDAC Substrate 2 (200 µM) to each well.

17. Incubate at 37°C for 30 minutes.

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

19. Incubate at room temperature for 15 minutes.

20. Immediately read in a fluorimeter or a microplate reader capable of excitation at λ=485 nm and detection at λ=528 nm.

21. The “Blank” value is subtracted from all other readings.

Component	**Blank**	**Positive Control**	**Control Inhibitor**	**HDAC Test Inhibitor**
Master Mix	35 µl	35 µl	35 µl	35 µl
Test Inhibitor	-	-	-	5 µl
Diluted Trichostatin A	-	-	5 µl	-
Diluent Solution	5 µl	5 µl	-	-
HDAC Assay Buffer	5 µl	-	-	-
Diluted HDAC2 (6 ng/µl) or Diluted HDAC of Interest	-	5 µl	5 µl	5 µl
Diluted HDAC Substrate 2 (200 µM)	5 µl	5 µl	5 µl	5 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

Example Results

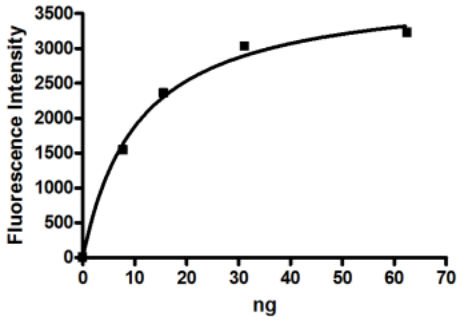

Figure 2: Fluorescence intensity measured in the presence of increasing HDAC2 concentrations. Fluorescence intensity was measured at λex=485 nm; λem=528 nm. The "Blank" value was subtracted from all other values.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References

Ito A., *et al.*, 2001 *EMBO J.* 20: 1331.
Barlev N.A, *et al.,* 2001 *Mol. Cell* 8: 1243.
Ito A., *et al.,* 2002 *EMBO J.* 21: 6236.

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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