Description

The rat histone deacetylase 6 (HDAC6) Assay Kit is a 96-well fluorogenic assay designed to measure rat HDAC6 enzymatic activity for screening and profiling applications. The kit contains enough purified HDAC6 protein, HDAC Assay Buffer, fluorogenic HDAC substrate and HDAC Developer solution for 100 reactions. In addition, the kit includes a potent HDAC inhibitor, Trichostatin A (TSA), for use as control.

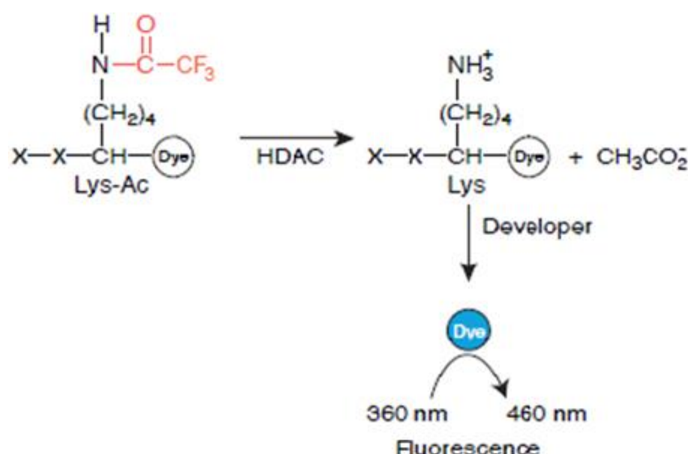

Figure 1: Illustration of the principle behind the Fluorogenic Rat HDAC Assay Kit.

The Fluorogenic Rat HDAC6 Assay Kit is based on a unique fluorogenic substrate and developer combination. A fluorescent dye is conjugated to a peptide containing acetyl-lysine. The fluorescence of the conjugated dye is quenched by the peptide. After treatment of the peptide with an HDAC, a developer solution specific for non-acetylated lysine is added to the reaction. If the acetyl group has been removed from the lysine, the developer releases the dye, allowing for fluorescence. In this assay, fluorescence directly correlates with HDAC activity.

Background

Histone Deacetylases (HDACs) catalyze the hydrolysis of an acetyl group from acetyl-lysine present in histones. Histone acetylation/deacetylation status tightly regulates transcription. HDAC6 plays a role in stress granule formation, cell motility, and leptin sensitivity. HDAC6 interacts with heat-shock protein 90 (HSP90) and with ubiquitinated proteins and has been linked to Alzheimer’s disease and tumor growth and metastasis. The use of selective inhibitors for HDAC6 offers potential benefits for neurodegenerative diseases and cancer treatment.

Applications

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

BPS Bioscience
Our products are for research use only, not for diagnostic or therapeutic use
bpsbioscience.com • 858-202-1401 • support@bpsbioscience.com

Supplied Materials

Catalog #	Name	Amount	Storage
101332	HDAC6, GST-His-Tag (Rat)*	9 µg	-80°C
50037	Fluorogenic HDAC substrate 3 (5 mM)	25 µl	-80°C
50030	2x HDAC Developer (contains Trichostatin A) (2 µM)	6 ml	-80°C
	Trichostatin A in DMSO (200 µM)**	100 µl	-20°C
50031	HDAC Assay Buffer	10 ml	-20°C
79685	Black 96-well plate	1	Room Temperature

**The concentration of the protein is lot-specific and will be indicated on the tube.*
***HDAC6 inhibitor Trichostatin A is provided as an internal control for HDAC6 activity.*

Materials Required but Not Supplied

- 1 mg/ml of bovine serum albumin (BSA) dissolved in distilled water
- Fluorescent microplate reader capable of reading λexc/λem=350-380 nm/440-460 nm
- Adjustable micropipettor and sterile tips

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.

Assay Protocol

- All samples and controls should be tested in duplicate.
- **If the assay plate is going to be used more than once, prepare enough reagents for this portion of the assay and store the remaining stock solutions into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C.**

1. Just before use, dilute **Trichostatin A (200 µM)** 10-fold with **HDAC Assay Buffer** to make a 20 µM solution. You will need 5 µl/well.

2. Dilute **Fluorogenic HDAC Substrate 3 (5 mM)** 25-fold with **HDAC Assay Buffer** to make a 200 µM solution. You will need 5 µl/well.

3. Dilute **HDAC6 Rat Recombinant Enzyme** in **HDAC Assay Buffer** to 7 ng/µl (5 µl/well). If not using all the protein, aliquot any remaining **undiluted** enzyme and store at -80°C. Keep the diluted enzyme on ice. Discard any remaining diluted enzyme after use.

   HDAC6 is sensitive to freeze/thaw cycles. Do not re-use thawed aliquots more than once and do not re-use the diluted protein.

Our products are for research use only, not for diagnostic or therapeutic use
BPS Bioscience ● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

4. Prepare a Master Mix as follows: N wells x (5 µl Fluorogenic HDAC substrate 3 (200 µM) + 5 µl BSA (1 mg/ml) + 30 µl HDAC Assay Buffer).

5. Add 40 µl of Master Mix to all wells.

6. Prepare 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.

   6.1. If the Test Inhibitor is soluble in water, make a dilution in HDAC Assay Buffer at a concentration 10-fold higher than the final desired concentration. The HDAC Assay Buffer is the Diluent Solution.
   OR
   6.2. If the Test Inhibitor is soluble in DMSO, dissolve in 100% DMSO at a concentration 100-fold higher than the highest desired concentration. Prepare a 10-fold dilution in HDAC Assay Buffer. The compound concentration is 10-fold higher than the final desired concentration and the concentration of DMSO is 10%.
   Prepare serial dilutions of the Test Inhibitor at concentrations 10-fold higher than the desired final concentrations using 10% DMSO in HDAC Buffer to keep the concentration of DMSO constant.
   For positive and negative controls, prepare 10% DMSO in HDAC Assay Buffer so that all wells contain the same amount of DMSO (Diluent Solution).

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

7. Add 5 µl of test inhibitor solution to each well designated “Test Inhibitor.”

8. Add 5 µl of diluted Trichostatin A solution to each well designated “Inhibitor Control.”

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

10. Add 5 µl HDAC Assay Buffer to the wells designated “Blank.”

11. Initiate the reaction by adding 5 µl of diluted **HDAC6 rat recombinant enzyme** to the wells designated “Positive Control,” “Test Inhibitor,” and “Inhibitor Control.”

Component	**Blank**	**Positive Control**	**Test Inhibitor**	**Inhibitor Control**
Master Mix	40 µl	40 µl	40 µl	40 µl
HDAC Assay Buffer	5 µl	-	-	-
Diluted Trichostatin A (20 µM)	-	-	-	5 µl
Test Inhibitor	-	-	5 µl	-
Diluent Solution	5 µl	5 µl	-	-
Rat HDAC6 (7 ng/µl)	-	5 µl	5 µl	5 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

12. Incubate at 37°C for 30 minutes.

Our products are for research use only, not for diagnostic or therapeutic use
BPS Bioscience
• bpsbioscience.com • 858-202-1401 • support@bpsbioscience.com

13. Add 50 µl of undiluted **2x HDAC Developer** to each well. Incubate the plate at room temperature for 15 minutes.

14. Read the plate using a fluorescence reader capable of excitation at a wavelength in the range of 350-380 nm and detection of emitted light in the range of 440-460 nm.

15. "Blank" value should be subtracted from all other values.

Example of Assay Results

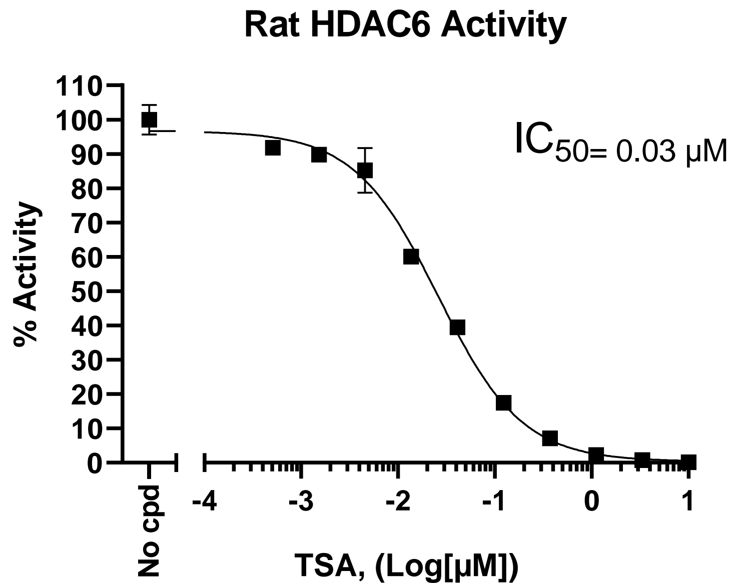

Figure 2: Inhibition of Rat HDAC6 Activity.
Rat HDAC6 activity was measured in the presence of increasing concentrations of Trichostatin A (TSA). Fluorescence was measured using a Bio-Tek fluorescent microplate reader. The "Blank" value was subtracted from all other values. Results are expressed as the percent of control activity (HDAC activity in the absence of inhibitor, set at 100%).

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

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

References
Hey J. et al., 2023, HDAC6 score: to treat or not to treat? *Nature Cancer*. 4: 156-158

Related Products

Products	*Catalog #*	*Size*
HDAC6, GST-Tag (HEK293-derived) Recombinant	100051	50 µg
HDAC6, GST-tag (Sf9-derived) Recombinant	50006	50 µg
HDAC6, GST-Tag (Mouse) Recombinant	50057	50 µg
HDAC11 Fluorogenic Assay Kit	50687	96 reactions
HDAC3 Fluorogenic Assay Kit	50073	96 reactions
HDAC Fluorogenic Assay Kit (Green)	50034	96 reactions

BPS Bioscience
Our products are for research use only, not for diagnostic or therapeutic use
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com