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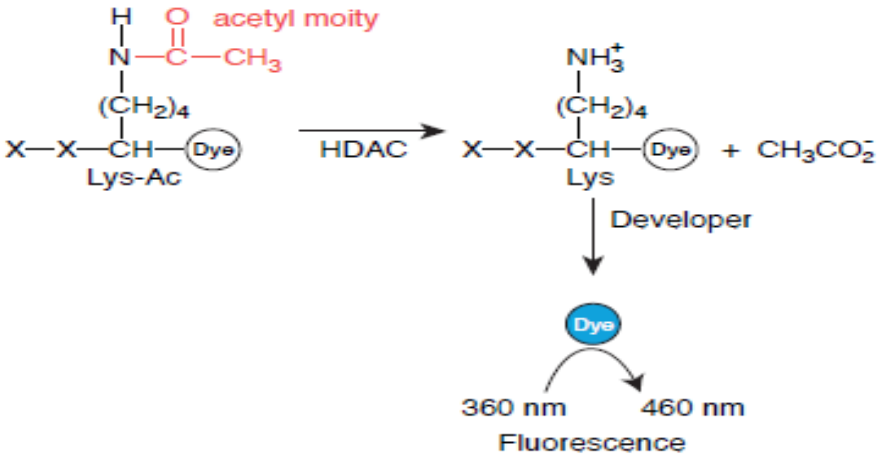
Data Sheet

Fluorogenic HDAC5 Assay Kit

Catalog #: 50065

DESCRIPTION: The *Fluorogenic HDAC5 Assay Kit* is a complete assay system designed to measure histone deacetylase 5 (HDAC5) activity for screening and profiling applications. It comes in a convenient 96-well format, with all the reagents necessary for 100 fluorescent HDAC5 activity measurements. In addition, the kit includes purified HDAC5 enzyme and a potent HDAC inhibitor, Trichostatin A, for use as a positive and negative control. The *Fluorogenic HDAC5 Assay Kit* is based on a unique fluorogenic substrate and developer combination. This assay method eliminates dealing with the radioactivity, extraction, and chromatography aspects of traditional assays. Using this kit, only two simple steps on a microtiter plate are needed to analyze the HDAC5 activity level. First, the HDAC fluorometric substrate, containing an acetylated lysine side chain, is incubated with purified HDAC5. The deacetylation sensitizes the substrate so subsequent treatment with the Lysine Developer produces a fluorophore that can then be measured using a fluorescence reader.

HDACs regulate cellular processes by catalyzing the hydrolysis of an acetyl group from acetyllysines in modified proteins. In the HDAC assay, fluorescent-dye molecules are attached to a peptide containing acetyllysine. Attachment to the peptide quenches the fluorescence of the dye. After treatment of the peptide with an HDAC, the reaction is mixed with a development solution that is specific for nonacetylated lysines. If the acetyl group has been removed from the lysine by the HDAC, this solution will release the dye allowing for fluorescence.



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COMPONENTS:

Cat. #	Component	Amount	Storage	
50005	HDAC5 human recombinant enzyme	1 µg	-80°C	
50040	Fluorogenic HDAC substrate class 2A (5 mM)	50 µl	-80°C	
50030	2x HDAC Developer (contains Trichostatin A) (50 µM)	6 ml	-80°C	***Avoid freeze/ thaw cycles!***
	Trichostatin A (1 mM) in DMSO	100 µl	-20°C	
50031	HDAC Assay Buffer	10 ml	-20°C	
79685	black, low binding NUNC black microtiter plate	1 plate	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
0.1% solution (1 mg/ml) of bovine serum albumin (BSA) in water
Fluorimeter capable of excitation at 350-380 nm and detection at 440-460 nm

APPLICATIONS: Great for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: One year from date of receipt when stored as directed.

REFERENCE(S): Ontoria, J.M., *et al., J. Med. Chem.* 2009 Nov 12;**52(21)**:6782-9.

ASSAY PROTOCOL:

Immediately prior to assay:

1) Dilute **Trichostatin A** 1 mM stock 10-fold with **HDAC Assay Buffer** to make a 100 µM solution. *Make only sufficient quantity needed for the assay; store remaining 1 mM **Trichostatin A** stock solution in aliquots at -80°C.*

2) Dilute **HDAC substrate** 5 mM stock 250-fold with **HDAC Assay Buffer** to make a 20 µM solution. (Make only sufficient quantity needed for the assay; store remaining 5 mM stock solution in aliquots at -80°C.)

3) Dilute HDAC5 in **HDAC Assay Buffer** to 0.6 ng/µl (3 ng/reaction)*. Aliquot any remaining enzyme and store undiluted at -80°C. Keep diluted enzyme on ice. Discard any remaining diluted enzyme after use. **Note: optimal enzyme concentration may vary with the specific activity of the enzyme.*

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Step 1:

In duplicate, add the reaction mixtures (below) to the microtiter black plate as follows:

1) Prepare the master mixture: N wells × (5 µl **HDAC substrate** (20 µM) + 5 µl BSA (1 mg/ml) + 30 µl **HDAC Assay Buffer**). Add 40 µl of master mixture to all wells.

2) Add 5 µl of inhibitor solution of each well designated “Test Inhibitor”. For the “Positive Control” and “Blank”, add 5 µl of the same solution without inhibitor (inhibitor buffer). Add 5 µl of diluted **Trichostatin A** (100 µM) to the well designated "Inhibitor Control". Keep final DMSO concentration at or below 1%.

3) Add 5 µl of **HDAC Assay Buffer** to the wells designated "Blank".

4) Initiate reaction by adding 5 µl of diluted **HDAC5 enzyme** to the wells designated “Positive Control”, "Test Inhibitor", and "Inhibitor Control". Incubate at 37ºC for 30 min.

	“Blank”	**Positive Control**	**Test Inhibitor**	**Inhibitor Control**
HDAC substrate (20 µM)	5 µl	5 µl	5 µl	5 µl
BSA (1 mg/ml)	5 µl	5 µl	5 µl	5 µl
HDAC Assay Buffer	35 µl	30 µl	30 µl	30 µl
Diluted Trichostatin A (100 µM)	–	–	–	5 µl
Test Inhibitor	–	–	5 µl	–
Inhibitor buffer (no inhibitor)	5 µl	5 µl	–	–
Diluted HDAC5 (0.6 ng/µl)	–	5 µl	5 µl	5 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

Step 2:
Add 50 µl of undiluted **HDAC Assay Developer (2x)** to each well. Incubate the plate at room temperature for 15 minutes.

Step 3:
Read sample in a microtiter plate-reading fluorimeter 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. “Blank” value is subtracted from all other values.

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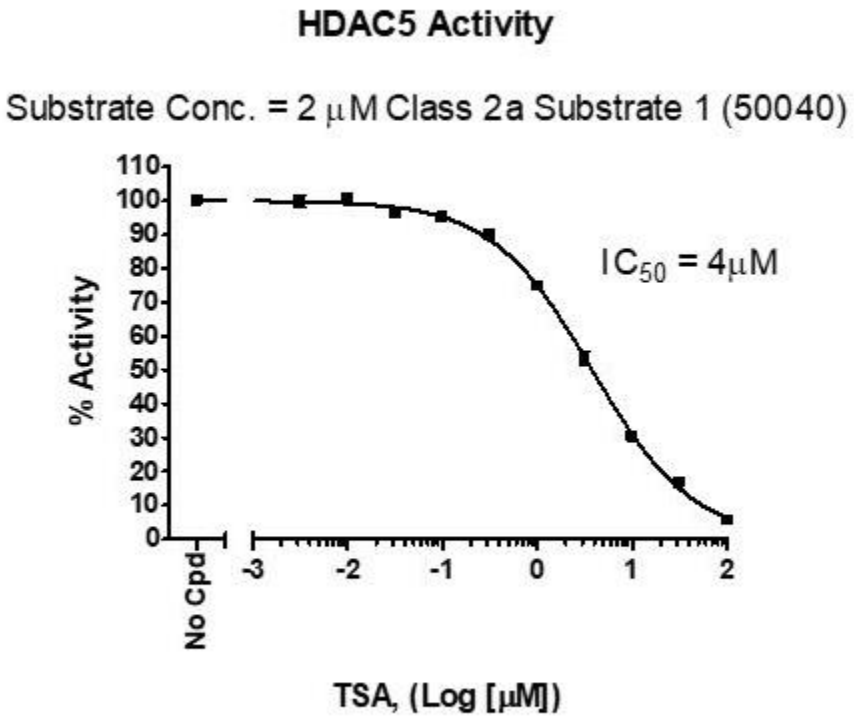
Example of Assay Results:



HDAC5 enzyme activity, measured using the *Fluorogenic HDAC5 Assay Kit*, BPS Bioscience Catalog #50065. Fluorescence was measured using a Bio-Tek fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at* info@bpsbioscience.com.

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RELATED PRODUCTS:

Product Name	Catalog #	Size
HDAC1	50051	50 µg
HDAC2 (C-His)	50002	50 µg
HDAC2 (C-Flag)	50052	50 µg
HDAC3/NcoR2	50003	50 µg
HDAC4	50004	10 µg
HDAC5	50005	10 µg
HDAC6 (C-Flag)	50056	50 µg
HDAC6 (N-GST)	50006	50 µg
HDAC6 (H216A)	50046	50 µg
HDAC6 (H611A)	50066	50 µg
HDAC7	50007	10 µg
HDAC8	50008	50 µg
HDAC9	50009	10 µg
HDAC10	50010	50 µg
HDAC11	50011	50 µg
HDAC Assay Kit	50033	96 reactions
HDAC Assay Kit (Green)	50034	96 reactions
HDAC Class 2a Assay Kit	50041	96 reactions
HDAC2 Assay Kit	50062	96 reactions
HDAC3 Assay Kit	50073	96 reactions
HDAC4 Assay Kit	50064	96 reactions
HDAC6 Assay Kit	50076	96 reactions
HDAC7 Assay Kit	50077	96 reactions
HDAC8 Assay Kit	50068	96 reactions
HDAC9 Assay Kit	50069	96 reactions

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