6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

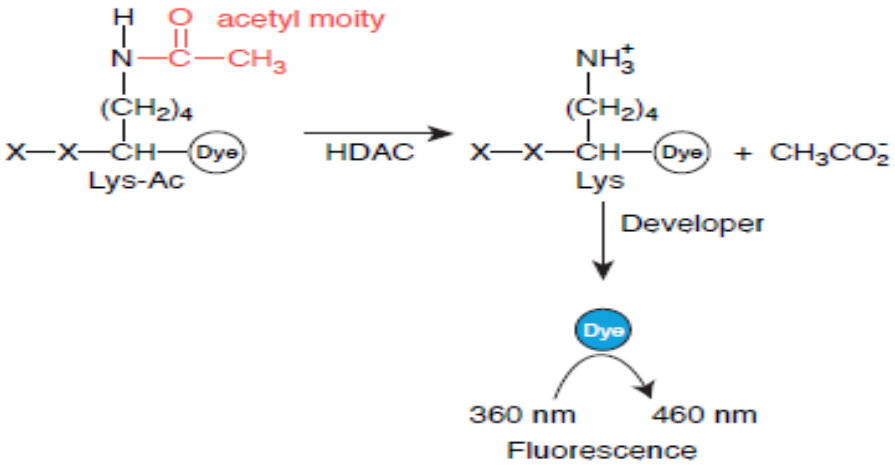
Data Sheet

HDAC6 Fluorogenic Assay Kit

Catalog #: 50076-2
384 Reactions

DESCRIPTION: The *Fluorogenic HDAC6 Assay Kit* is a complete assay system designed to measure histone deacetylase 6 (HDAC6) activity for screening and profiling applications. It comes in a convenient 384-well format, with all the reagents necessary for 384 fluorescent HDAC6 activity measurements, including purified HDAC6 enzyme. The *Fluorogenic HDAC6 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 HDAC activity level. First, the HDAC fluorometric substrate, containing an acetylated lysine side chain, is incubated with purified HDAC enzyme. 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 fluorescence. Fluorescence is therefore directly related to HDAC activity.



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COMPONENTS:

Catalog #	Reagent	Amount	Storage	
50006	HDAC6 human recombinant enzyme	9 µg	-80°C	***Avoid Freeze/ Thaw Cycles!***
50037	Fluorogenic HDAC substrate 3 (5 mM)	50 µl	-80°C	
50030	2x HDAC Developer (contains Trichostatin A) (50 µM)	10 ml	-80°C	
50031	HDAC Assay Buffer	10 ml	-20°C	
	384-well black, low binding microtiter plate	1	Room temp.	

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

MATERIALS 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.
Adjustable micropipettor and sterile tips
Rotating or rocker platform

REFERENCE(S):

1. Santo, L., *et al., Blood.* 2012 Mar 15;**119(11)**:2579-89.
2. Bradner, J.E., *et al., Nat Chem Biol.* 2010 Mar;**6(3)**: 238-243.

ASSAY PROTOCOL:

Immediately prior to assay:

1) Dilute **Fluorogenic HDAC substrate 3 (5 mM)** stock 25-fold with **HDAC Assay Buffer** to make a 200 µM solution. (Make only sufficient quantity needed for the assay; store remaining 5 mM stock solution in aliquots at -80°C).

2) Dilute **HDAC6 human recombinant enzyme** in **HDAC Assay Buffer** to 7 ng/µl (17.5 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.*

Step 1:
In duplicate, add the reaction mixtures (below) to the microtiter black plate as follows:

1) Prepare the master mixture: N wells × (2.5 µl diluted **Fluorogenic HDAC substrate 3 (200 µM)** + 2.5 µl BSA (1 mg/ml) + 15 µl **HDAC Assay Buffer**). Add 20 µl of master mixture to all wells.

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2) Prepare the inhibitor.

The final concentration of DMSO in the assay should not exceed 1%. If the inhibitor compound is dissolved in DMSO, make a 100-fold higher concentration of the compound than the highest concentration you want to test in DMSO. Then make a 10-fold dilution in distilled water (at this step the compound concentration is 10-fold higher than the final concentration).

If the inhibitor compound is dissolved in water, make a solution of the compound 10-fold higher than the final testing concentration.

3) Add 2.5 µl of inhibitor to each well designated “Test Inhibitor.” For the “Positive Control” and “Blank,” add 2.5 µl of the same solution without inhibitor (either water or 10% DMSO in water,depending which inhibitor solution is used). Keep final DMSO concentration at or below 1%.

4) Add 2.5 µl of **HDAC Assay Buffer** to the wells designated "Blank."

5) Initiate reaction by adding 2.5µl of diluted **HDAC6 human recombinant enzyme** to the wells designated “Positive Control,” "Test Inhibitor," and "Inhibitor Control." Incubate at 37ºC for 30 minutes.

	“Blank”	Positive Control	Test Inhibitor
HDAC substrate (200 µM)	2.5 µl	2.5 µl	2.5 µl
BSA (1 mg/ml)	2.5 µl	2.5 µl	2.5 µl
HDAC Assay Buffer	17.5 µl	15 µl	15 µl
Test Inhibitor	–	–	2.5 µl
10% DMSO in water (Inhibitor buffer)	2.5 µl	2.5 µl	–
Diluted HDAC6 (7 ng/µl)	–	2.5 µl	2.5 µl
Total	25 µl	25 µl	25 µl

Step 2:

Add 25 µl of undiluted **2x HDAC Developer** 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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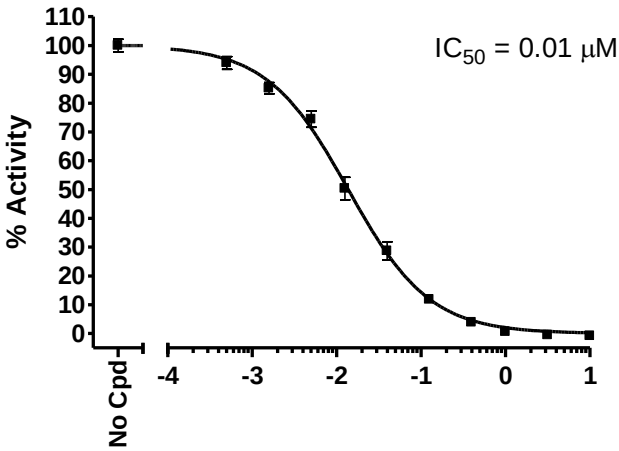
Example of Assay Results:

HDAC6 Activity, 384 well

Substrate Conc.=10 μM Substrate 3 (50037)



HDAC6 enzyme activity, measured using the *HDAC6 Fluorogenic Assay Kit*, BPS Bioscience Catalog #50076. 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.*

RELATED PRODUCTS:

Product	Catalog #	Size
HDAC6 Fluorogenic Assay Kit	50076-1	96 rxns
HDAC6 (C-Flag)	50056	50 µg
HDAC6 (N-GST)	50006	50 µg
HDAC6 (H216A)	50046	50 µg
HDAC6 (H611A)	50066	50 µg
HDAC1, FLAG-tag, His-tag	50051	50 µg
HDAC2, FLAG-tag	50052	50 µg
HDAC3/NcoR2 Complex, His-tag	50003	50 µg
HDAC4, GST-tag, His-tag	50004	10 µg
HDAC5, Catalytic Domain, His-tag	50005	10 µg
HDAC7, GST-tag	50007	10 µg
HDAC8, His-tag	50008	50 µg
HDAC9, His-tag	50009	10 µg
HDAC10, GST-tag	50010	50 µg
HDAC11, GST-tag	50011	50 µg
HDAC Fluorogenic Assay Kit	50033	96 rxns
HDAC Class 2a Fluorogenic Assay Kit	50041	96 rxns