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Data Sheet

Fluorogenic HDAC Assay Kit (Green)

Catalog #: 50034

DESCRIPTION: The *Fluorogenic HDAC Assay Kit (Green)* is a complete assay system designed to measure histone deacetylase (HDAC) class 1 activity for screening and profiling applications. The kit includes a specific HDAC substrate that emits light within the green light spectrum (ex 485 nm/em 528 nm)*.

**Note: This kit is particularly suitable if the researcher's test inhibitor sample(s) fluoresce in the same range as the substrates in our other HDAC assay kits, ex 350-380 nm/em 440-460 nm. If inhibitor fluorescence is not an issue, the Fluorogenic HDAC Assay Kit, catalog #50033 is recommended.*

The *Fluorogenic HDAC Assay Kit (Green)* comes in a convenient 96-well format, with all the reagents necessary for 100 fluorescent HDAC activity measurements. In addition, the kit includes purified HDAC2 enzyme and a potent HDAC inhibitor, Trichostatin A, for use as a positive and negative control. The *Fluorogenic HDAC Assay Kit (Green)* 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 at 485 nm (excitation)/528 nm (emission).

COMPONENTS:

Cat. #	Component	Amount	Storage	Storage
50002	HDAC2 human recombinant enzyme	6 µg	-80°C	***Avoid freeze/ thaw cycles!***
50038	Fluorogenic HDAC substrate 2 (5 mM)	50 µl	-80°C	
50030	2x HDAC Developer (contains Trichostatin A) (50 µM)	6 ml	-80°C	
	Trichostatin A (200 µM)	100 µl	-20°C	
50031	HDAC assay buffer	10 ml	-20°C	
	black, low binding NUNC black microtiter plate	1 plate	Room temp.	

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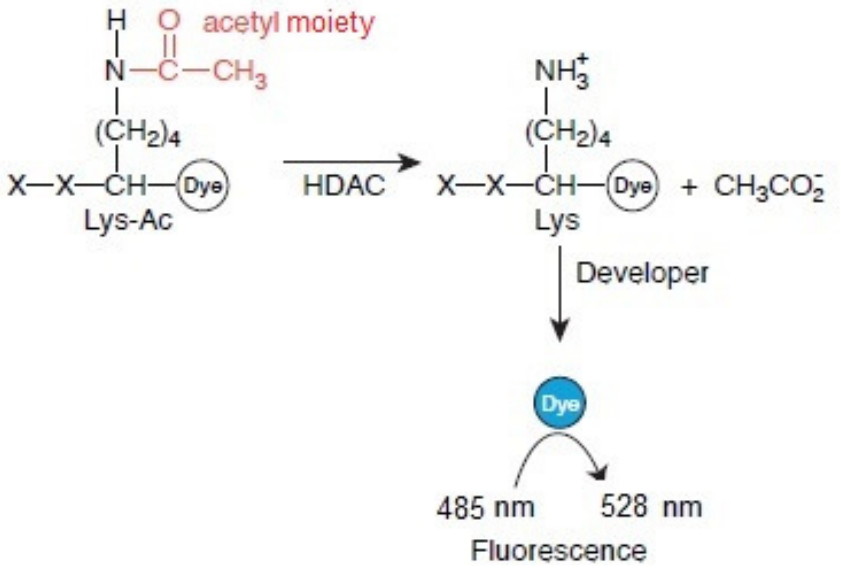
APPLICATIONS: Great for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications, using samples that may fluoresce within the 440-460 nm spectrum. This kit is suitable for class 1 and class 2b HDACs (HDACs 1, 2, 3, 6, 10).

BACKGROUND: 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. Fluorescence is therefore directly related to HDAC activity.



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

REFERENCES:

1. A. Ito *et al.* (2001) *EMBO J.* **20** 1331.
2. N.A. Barlev *et al.* (2001) *Mol. Cell* **8** 1243.
3. A. Ito *et al.* (2002) *EMBO J.* **21** 6236.

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ASSAY PROTOCOL:

Immediately prior to assay:

1) Dilute HDAC substrate 2 (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 HDAC2 in HDAC assay buffer to 6 ng/µl (30 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:
Perform all reactions in duplicate.

Component	Positive Control	Inhibitor Control	Test Inhibitor	Blank
HDAC substrate 2 (200 µM)	5 µl	5 µl	5 µl	5 µl
BSA (1 mg/ml)	5 µl	5 µl	5 µl	5 µl
HDAC assay buffer	30 µl	30 µl	30µl	35 µl
Trichostatin A	–	5 µl	–	–
Test Inhibitor	–	–	5 µl	–
Inhibitor Buffer (no inhibitor)	5 µl			5 µl
HDAC2 (6 ng/µl)	5 µl	5 µl	5 µl	–
Total	50 µl	50 µl	50 µl	50 µl

Add the reaction mixtures to the black microtiter plate as follows:

1) Prepare the master mixture: N wells x (5 µl HDAC substrate (200 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”.

3) For the “Positive Control" and “Blank”, add 5 µl of the same solution without inhibitor (inhibitor buffer).

4) Add 5 µl of Trichostatin A (200 µM) to the wells designated “Inhibitor Control”.

5) Add 5 µl of HDAC **assay buffer** to the wells designated "Blank".

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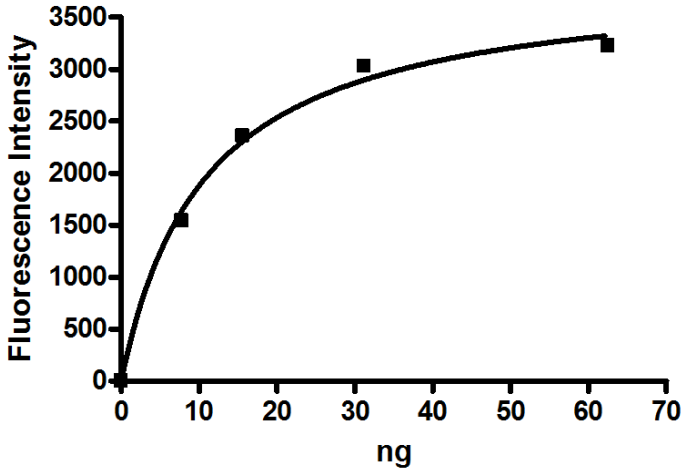
6) Initiate reaction by adding 5 µl of diluted **HDAC2 enzyme** to the wells designated "Positive Control", "Inhibitor Control", and "Test Inhibitor Control". Incubate at 37ºC for 30 min.

Step 2:
Add 50 µl of 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 ~485 nm and detection of emitted light in the range of 528 nm. "Blank" value is subtracted from all other values.

Example of Assay Results:



HDAC2 enzyme activity, measured using the Fluorogenic HDAC Assay Kit (Green), BPS Bioscience #50034. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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RELATED PRODUCTS

Fluorogenic HDAC Assay Kit	#50033	96 rxns.
Fluorogenic HDAC Class 2a Assay Kit	#50041	96 rxns.
Fluorogenic HDAC8 Assay Kit	#50068	96 rxns.
HDAC Assay Buffer	#50031	20 mL
HDAC Assay Developer	#50030	6 mL
HDAC1 Enzyme	#50051	50 µg
HDAC2 Enzyme	#50002	50 µg
HDAC3 Enzyme	#50003	50 µg
HDAC4 Enzyme	#50004	10 µg
HDAC5 Enzyme	#50005	10 µg
HDAC6 Enzyme	#50006	50 µg
HDAC7 Enzyme	#50007	10 µg
HDAC8 Enzyme	#50008	50 µg
HDAC9 Enzyme	#50009	10 µg
HDAC10 Enzyme	#50010	50 µg
HDAC11 Enzyme	#50010	50 µg