

Data Sheet***Fluorogenic HDAC Class2a Assay Kit*****Catalog #: 50041**

Lot# 2000

DESCRIPTION: The *Fluorogenic HDAC Class2a Assay Kit* is a complete assay system designed to measure histone deacetylase (HDAC) class2a activity in cell extracts, nuclear extracts, immunoprecipitates, and purified enzymes. It comes in a convenient 96-well format, with all the reagents necessary for 100 fluorescent HDAC activity measurements. In addition, the kit includes purified HDAC4 and a potent HDAC inhibitor, Trichostatin A, for use as a positive and negative control. The *Fluorogenic HDAC Class 2a 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 class2a activity level. First, the HDAC fluorometric substrate is incubated with a sample containing HDAC activity (e.g. HeLa nuclear extract or purified HDAC4 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.

COMPONENTS:

	Cat. #	Lot #	Reagent	Amount	Storage
<i>Avoid freeze/thaw cycles!</i>	50004		HDAC4 human recombinant enzyme	0.1 µg	-80°C
	50040		Fluorogenic HDAC substrate (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.

APPLICATIONS: Great for studying enzyme kinetics and screening small molecular inhibitors of class2a HDACs for drug discovery and HTS applications.

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ASSAY PROTOCOL:

Immediately prior to assay:

- 1) Dilute HDAC substrate 5 mM stock 25-fold with HDAC assay buffer to make a 200 μ M 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 0.2 ng/ μ l (1 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. Incubate at 37°C for 30 min.

	Enzyme Positive Control	Inhibitor Negative Control	Test Inhibitor	“Blank” Negative Control
HDAC4 (0.2 ng/ μ l)	5 μ l	5 μ l	5 μ l	–
HDAC substrate (200 μ M)	5 μ l	5 μ l	5 μ l	5 μ l
BSA (1 mg/ml)	5 μ l	5 μ l	5 μ l	5 μ l
Trichostatin A	–	5 μ l	–	–
Test Inhibitor	–	–	X μ l	–
HDAC assay buffer	35 μ l	30 μ l	35 - X μ l	40 μ l
Total	50 μl	50 μl	50 μl	50 μl

Step2:

Add 50 μ l 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 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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