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Data Sheet

Fluorogenic HDAC Class2a Assay Kit

Catalog #: w60052

DESCRIPTION: The *Fluorogenic HDAC Class2a Assay Kit* is a complete assay system designed to measure histone deacetylase (HDAC) class2a activity for screening and profiling applications. 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 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:

Catalog #	Component	Amount	Storage	
w60015	HDAC4 human recombinant enzyme	1 µg	-80°C	***Avoid freeze/ thaw cycles!***
w60051	Fluorogenic HDAC substrate class 2A (5 mM)	50 µl	-80°C	
w60041	2x HDAC Developer (contains Trichostatin A) (50 µM)	6 ml	-80°C	
	Trichostatin A (1 mM) in DMSO	100 µl	-20°C	
w60042	HDAC assay buffer	10 ml	-20°C	
	black, low binding NUNC black microtiter plate	1 plate	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
1% solution (10 µg/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

APPLICATIONS: Great for studying enzyme kinetics and screening small molecular inhibitors of class2a HDACs for drug discovery and HTS applications. This kit is suitable for Class 2a and class 4 HDACs (HDACs 4, 5, 7, 9, 11). For class 1 and class 2b HDACs (HDACs HDACs 1, 2, 3, 6, 10), we recommend the Fluorogenic HDAC Assay

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Kits, Cat. #w60044 or #w60045, or for HDAC8, we recommend the Fluorogenic HDAC8 Assay Kit, Cat. # w60079.

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 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.)

3) Dilute **HDAC4** in **HDAC Assay Buffer** to 12 pg/µl (60 pg/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 × (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”. 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 wells 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 **HDAC4 enzyme** to the wells designated “Positive Control”, "Test Inhibitor", and "Inhibitor Control". Incubate at 37ºC for 30 min.

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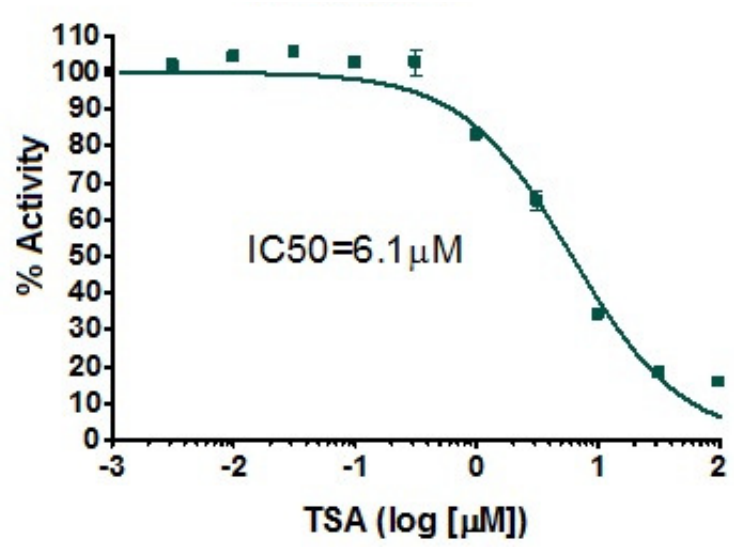
	"Blank"	Positive Control	Test Inhibitor	Inhibitor Control
HDAC substrate (200 µM)	5 µl	5 µl	5 µl	5 µl
BSA (1 mg/ml)	5 µl	5 µl	5 µl	5 µl
HDAC Assay Buffer	35 µl	30 µl	30 µl	30 µl
Diluted Trichostatin A (100 µM)	–	–	–	5 µl
Test Inhibitor	–	–	5 µl	–
Inhibitor buffer (no inhibitor)	5 µl	5 µl	–	–
Diluted HDAC4 (0.012 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 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.

Example of Assay Results:



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HDAC4 enzyme activity, measured using the *Fluorogenic HDAC4 Assay Kit*, West Bioscience Catalog #w60075. Fluorescence was measured using a Bio-Tek fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact West Bioscience, Inc. at* sale@westbioscience.com.