WEST BIOSCIENCE

9891 Irvine Center Dr. Suite 200
Irvine, CA 92618
United States
Tel: 1.800.831.1518
Fax: 1.800.831.1518
Email: sale@westbioscience.com

Data Sheet

Kinetic HDAC1 Assay Kit
Catalog #: w63012

DESCRIPTION: The *Kinetic HDAC1 Assay Kit* is a complete assay system designed to measure histone deacetylase 1 (HDAC1) activity kinetically for screening and profiling applications. It comes in a convenient 96-well format, with all the reagents necessary for 100 fluorescent HDAC1 activity measurements. In addition, the kit includes purified HDAC1 enzyme and a potent HDAC inhibitor, SAHA, for use as a positive and negative control. The *Kinetic HDAC1 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 one simple step for setting up the HDAC1 reaction on a microtiter plate is needed and then the HDAC1 activity level can be measured kinetically using a fluorescence reader.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
w60062	HDAC1 human recombinant enzyme	10 µg	-80°C	***Avoid Freeze/ Thaw Cycles!***
w60048	Fluorogenic HDAC substrate (5 mM)	50 µl	-80°C	
w63040	10x HDAC Developer	1 ml	-80°C	
	Developer Dilution Buffer	10 ml	-20°C	
	SAHA (1 mM)	20 µl	-20°C	
w60042	HDAC Assay Buffer	20 ml	-20°C	
	BSA (1mg/ml)	1 ml	-20°C	
	black, low binding NUNC black microtiter plate	1 plate	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader

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):

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.

WEST BIOSCIENCE

9891 Irvine Center Dr. Suite 200
Irvine, CA 92618
United States
Tel: 1.800.831.1518
Fax: 1.800.831.1518
Email: sale@westbioscience.com

ASSAY PROTOCOL:

Thaw all of the components except the HDAC enzyme, and place on ice. All of the reactions should be set up on ice.

1) Prepare the master **HDAC Substrate Solution**
   To make the master **HDAC Substrate Solution,** mix 2,368 µl **HDAC Assay Buffer,** 800 µl BSA (1 mg/ml), and 32 µl **HDAC Substrate.** This is enough for 100 reactions. If you have less than 100 reactions, you can reduce the components proportionally. (Make sufficient quantity needed for the assay; store remaining BSA and 5 mM stock solution in aliquots at -80°C.)

   Add 20 µl of the master **HDAC Substrate Solution** to each well.

2) Prepare **Inhibitor Solutions**
   Dilute the Test Inhibitor 10-fold higher than the final concentration you want to test in 10% DMSO (the final DMSO concentration is 1% in all of the reactions).

   Dilute the same buffer without the inhibitor (Inhibitor Buffer) in 10% DMSO as a control.

   Add 10 µl of the **Test Inhibitor Solution** to each well designated as "Test Inhibitor", and 10 µl of **SAHA Solution** to each well designated "SAHA Inhibitor Control". Add 10 µl of the Inhibitor Buffer to each well designated "Blank" and "Positive Control".

3) Prepare **Developer Solution**
   Dilute the **10X Developer Solution** (1:10 dilution) in **Developer Dilution Buffer.** Add 50 µl of the **Developer Solution** to each well.

4) Prepare **HDAC1 Enzyme Solution**
   Dilute **HDAC1** in HDAC assay buffer to 4 ng/µl (80 ng/reaction). Aliquot any remaining enzyme and store undiluted at -80°C.

   Add 20 µl of HDAC assay buffer to each well designed "Blank". Add 20 µl of the **HDAC1 enzyme solution** to each well designed "Positive Control", "Test Inhibitor" and "SAHA Inhibitor Control". **Always add the enzyme solution last.**

5) Measure the plate in 5 minute intervals for a period of up to one hour, using a microtiter plate-reading fluorimeter capable of excitation in the range of 350-380 nm and detection of emitted light in the range of 440-460 nm. The "Blank" value is subtracted from all other values.

WEST BIOSCIENCE

9891 Irvine Center Dr. Suite 200
Irvine, CA 92618
United States
Tel: 1.800.831.1518
Fax: 1.800.831.1518
Email: sale@westbioscience.com

	"Blank"	Positive Control	Test Inhibitor	SAHA Inhibitor Control
Master HDAC Substrate solution	20 µl	20 µl	20 µl	20 µl
SAHA	–	–	–	10 µl
Test Inhibitor	–	–	10 µl	–
Inhibitor buffer (no inhibitor)	10 µl	10 µl	–	
1X Developer	50 µl	50 µl	50 µl	50 µl
HDAC assay buffer	20 µl	–	–	–
HDAC1 (4 ng/µl)	–	20 µl	20 µl	20 µl
Total	100 µl	100 µl	100 µl	100 µl

Example of Assay Results:

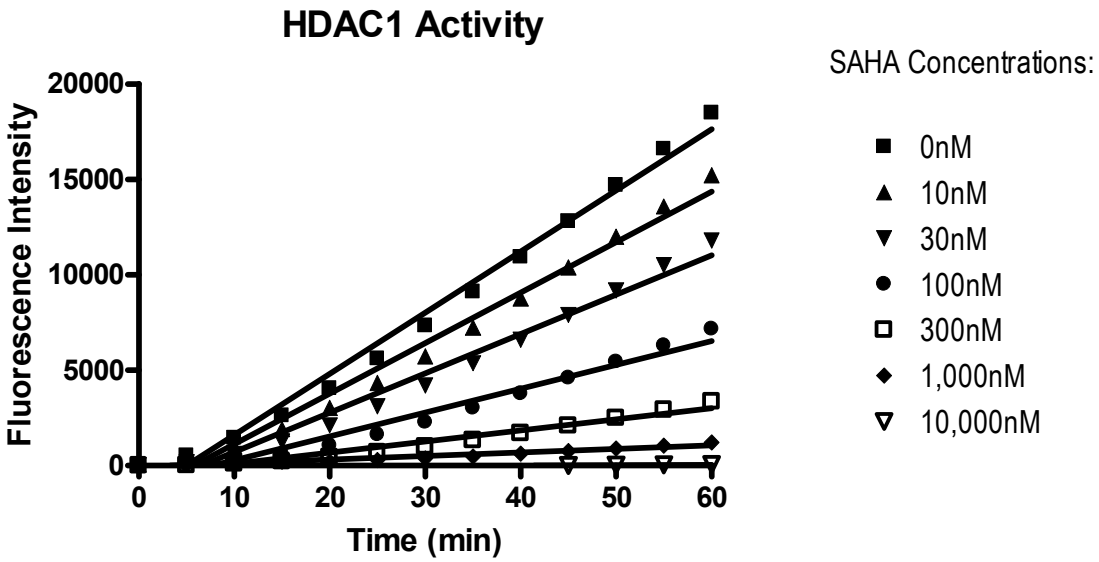

Figure 1. Time course of HDAC1 activity against different concentrations of SAHA, measured using the *Kinetic HDAC1 Assay Kit*, West Bioscience Cat# w63012. Fluorescence was measured using a Tecan fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact West Bioscience, Inc. at sale@westbioscience.com.*

9891 Irvine Center Dr. Suite 200
Irvine, CA 92618
United States
Tel: 1.800.831.1518
Fax: 1.800.831.1518
Email: sale@westbioscience.com

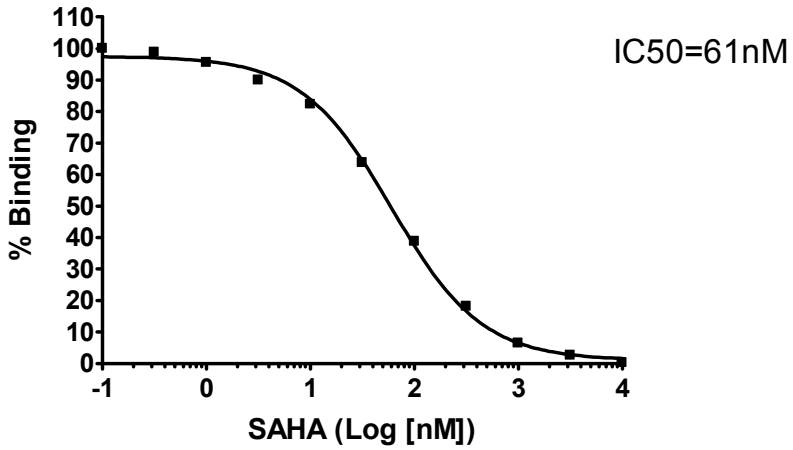

Figure 2. IC50 Assay for HDAC1 against SAHA.