

## Data Sheet

### ***Kinetic HDAC3 Assay Kit***

Catalog #: w63014

**DESCRIPTION:** The *Kinetic HDAC3 Assay Kit* is a complete assay system designed to measure histone deacetylase 3 (HDAC3) activity kinetically for screening and profiling applications. It comes in a convenient 96-well format, with all the reagents necessary for 100 fluorescent HDAC3 activity measurements. In addition, the kit includes purified HDAC3/NCOR2 enzyme complex and a potent HDAC inhibitor, SAHA, for use as a positive control. The *Kinetic HDAC3 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 HDAC3 reaction on a microtiter plate is needed and then the HDAC3 activity level can be measured kinetically using a fluorescence reader.

#### **COMPONENTS:**

Catalog #	Reagent	Amount	Storage	
w60014	HDAC3/NCOR2 human recombinant enzyme	2 µg	-80°C	<b>Avoid Freeze/Thaw Cycles!</b>
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.

#### **ASSAY PROTOCOL:**

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

**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 2368  $\mu$ l **HDAC Assay Buffer**, 800  $\mu$ l BSA (1mg/ml), and 32  $\mu$ l **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  $-80^{\circ}\text{C}$ .)

Add 20  $\mu$ l of the master **HDAC Substrate Solution** to each well.

2) Prepare **Inhibitor Solutions**

Dilute the Test Inhibitor in 10% DMSO at 10-fold higher than the final concentration you want to test (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  $\mu$ l of the **Test Inhibitor Solution** to each well designated as "Test Inhibitor", and 10  $\mu$ l of **SAHA Solution** to each well designated as "SAHA Inhibitor Control". Add 10  $\mu$ l 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  $\mu$ l of the **Developer Solution** to each well.

4) Prepare **HDAC3/NCOR2 Enzyme Solution**

Dilute **HDAC3/NCOR2** in HDAC assay buffer to 0.8 ng/ $\mu$ l (16 ng/reaction). Aliquot any remaining enzyme and store undiluted at  $-80^{\circ}\text{C}$ .

Add 20  $\mu$ l of HDAC assay buffer to each well designed "Blank". Add 20  $\mu$ l of the **HDAC3/NCOR2 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.

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	“Blank”	Positive Control	Test Inhibitor	SAHA Inhibitor Control
Master HDAC Substrate Solution	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l
SAHA	-	-	-	10 $\mu$ l
Test Inhibitor	-	-	10 $\mu$ l	-
Inhibitor buffer (no inhibitor)	10 $\mu$ l	10 $\mu$ l	-	-
1X Developer	50 $\mu$ l	50 $\mu$ l	50 $\mu$ l	50 $\mu$ l
HDAC assay buffer	20 $\mu$ l	-	-	-
HDAC3/NCOR2 (0.8 ng/ $\mu$ l)	-	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l
Total	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l

## Example of Assay Results:

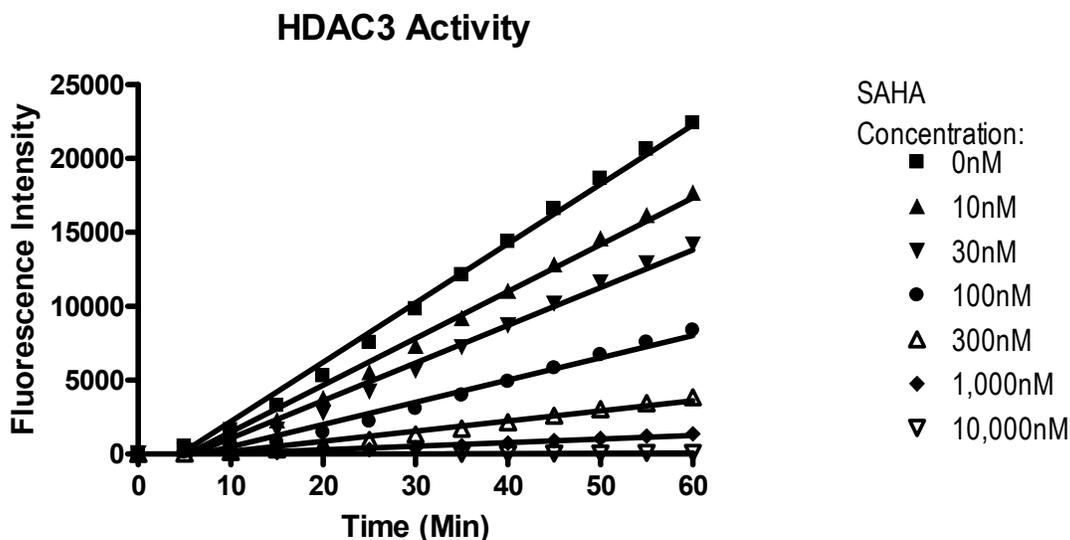


Figure 1. Time course of HDAC3 activity against different concentration of SAH. HDAC3 activity is measured using the *Kinetic HDAC3 Assay Kit*, West Bioscience Cat# w63014. Fluorescence was measured using a Tecan fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact West Bioscience, Inc. at [sale@westbioscience.com](mailto:sale@westbioscience.com).*

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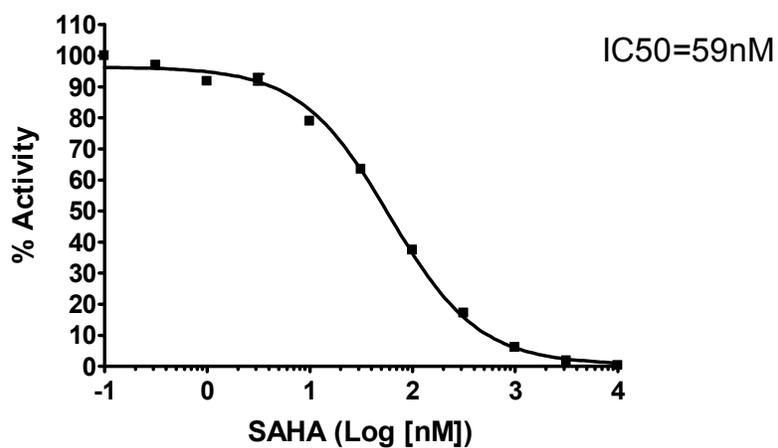


Figure 2. IC50 assay for HDAC3 against SAHA.