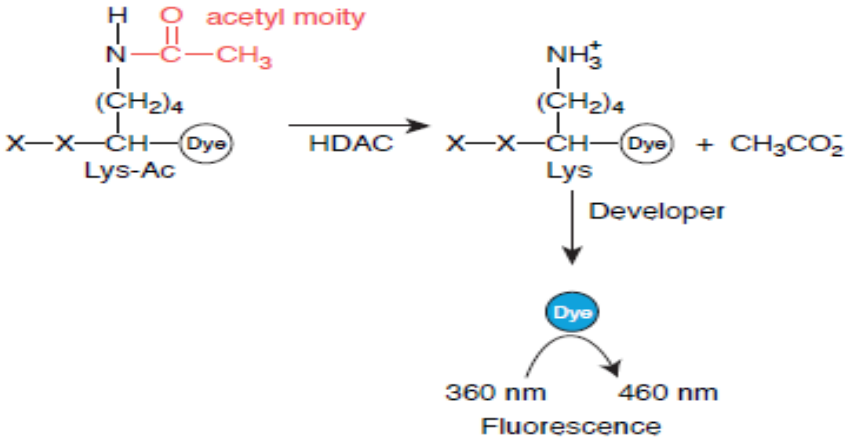
Data Sheet

Fluorogenic HDAC1 Assay Kit

Catalog #: AMS.50061

DESCRIPTION: *The Fluorogenic HDAC1 Assay Kit is a complete assay system designed to measure histone deacetylase 1 (HDAC1) activity 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, Trichostatin A, for use as a positive and negative control. The Fluorogenic 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 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 HDAC1. The deacetylation sensitizes the substrate so subsequent treatment with the Lysine Developer produces a fluorophore that can then be measured using a fluorescence reader.*

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.



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COMPONENTS:

Catalog #	Reagent	Amount	Storage	
50051	*HDAC1 human recombinant enzyme*	*1 µg*	*-80°C*	***Avoid Freeze/ Thaw Cycles!***
50037	*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.*	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
BSA (bovine serum albumin) (1 mg/ml)
Fluorescent microplate reader
Adjustable micropipettor and sterile tips
Rotating or rocker platform

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:

Immediately prior to assay:

1) *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.)*

2) *Dilute* **HDAC1** *in* **HDAC assay buffer** *to 0.4 ng/µl (2 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 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", "Inhibitor Buffer Control" and "Blank", add 5 µl of the same solution without inhibitor (inhibitor buffer). Add 5 µl of* **Trichostatin A** *(200 µM) to the well designated "TSA Inhibitor Control".*

	"Blank"	**Positive Control**	**Inhibitor Buffer Control**	**Test Inhibitor**	**TSA Inhibitor Control**
HDAC substrate (200 µM)	*5 µl*	*5 µl*	*5 µl*	*5 µl*	*5 µl*
BSA (1 mg/ml)	*5 µl*	*5 µl*	*5 µl*	*5 µl*	*5 µl*
HDAC assay buffer	*35 µl*	*30 µl*	*30 µl*	*30 µl*	*30 µl*
Trichostatin A	–	–		–	*5 µl*
Test Inhibitor	–	–	–	*5 µl*	–
Inhibitor buffer (no inhibitor)	*5 µl*	*5 µl*	*5 µl*	–	
HDAC1 (0.4 ng/µl)	–	*5 µl*	*5 µl*	*5 µl*	*5 µl*
Total	*50 µl*	*50 µl*	*50 µl*	*50 µl*	*50 µl*

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

4) *Initiate reaction by adding 5 µl of diluted HDAC1 enzyme to the wells designated "Positive Control", "Inhibitor Buffer Control", "Test Inhibitor", and "TSA Inhibitor Control". Incubate at 37ºC for 30 min.*

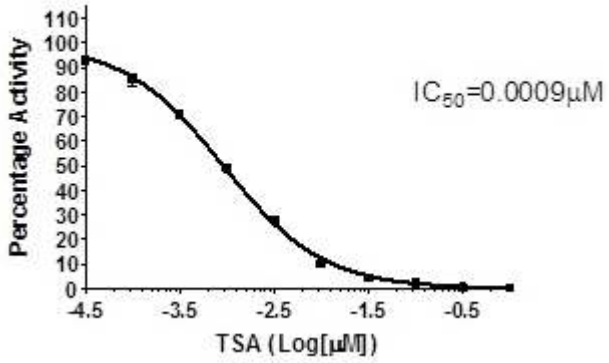
Step 2:

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 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:



HDAC1 enzyme activity, measured using the HDAC1 Fluorogenic Assay Kit, #AMS.40061. Fluorescence was measured using a Bio-Tek fluorescentmicroplate reader. Data shown is lot-specific.

RELATED PRODUCTS:

Product Name	Catalog #	Size
HDAC1	*50051*	*50 µg*
HDAC2 (C-His)	*50002*	*50 µg*
HDAC2 (C-Flag)	*50052*	*50 µg*
HDAC3/NcoR2	*50003*	*50 µg*
HDAC4	*50004*	*10 µg*
HDAC5	*50005*	*10 µg*
HDAC6 (C-Flag)	*50056*	*50 µg*
HDAC6 (N-GST)	*50006*	*50 µg*
HDAC6 (H216A)	*50046*	*50 µg*
HDAC6 (H611A)	*50066*	*50 µg*
HDAC7	*50007*	*10 µg*
HDAC8	*50008*	*50 µg*
HDAC9	*50009*	*10 µg*
HDAC10	*50010*	*50 µg*
HDAC11	*50011*	*50 µg*
HDAC Assay Kit	*50033*	*96 reactions*

HDAC Assay Kit (Green)	*50034*	*96 reactions*
HDAC Class 2a Assay Kit	*50041*	*96 reactions*
HDAC2 Assay Kit	*50062*	*96 reactions*
HDAC3 Assay Kit	*50073*	*96 reactions*
HDAC6 Assay Kit	*50076*	*96 reactions*
HDAC8 Assay Kit	*50068*	*96 reactions*

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