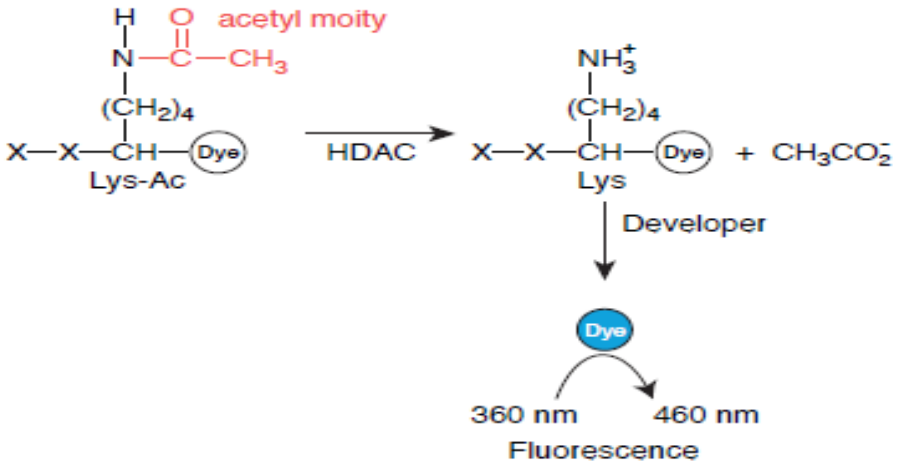
Data Sheet

Fluorogenic HDAC6 Assay Kit

Catalog #: AMS.50076

DESCRIPTION: The *Fluorogenic HDAC6 Assay Kit* is a complete assay system designed to measure histone deacetylase 6 (HDAC6) activity for screening and profiling applications. It comes in a convenient 96-well format, with all the reagents necessary for 100 fluorescent HDAC6 activity measurements. In addition, the kit includes purified HDAC6 enzyme and a potent HDAC inhibitor, Trichostatin A for use as a positive and negative control. The *Fluorogenic HDAC6 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 HDAC 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.

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	
50006	HDAC6 human recombinant enzyme	9 µg	-80℃	***Avoid Freeze/ Thaw Cycles!***
50037	Fluorogenic HDAC substrate (5 mM)	50 µl	-80℃	
50030	2x HDAC Developer (contains Trichostatin A) (50 µM)	6 ml	-80℃	
	Trichostatin A in DMSO (200 µM)	100 µl	-20℃	
50031	HDAC Assay Buffer	10 ml	-20℃	
	black, low binding NUNC black microtiter plate	1	Room temp.	

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

MATERIALS REQUIRED BUT NOT SUPPLIED:
0.1% solution (1 mg/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

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 **Trichostatin A in DMSO (200 µM)** stock 10-fold with **HDAC Assay Buffer** to make a 20 µM solution. (Make only sufficient quantity needed for the assay; store remaining **Trichostatin A in DMSO (200 µM)** stock solution in aliquots at -80℃.)

2) Dilute **Fluorogenic 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 2.5 mM stock solution in aliquots at -80℃.)

3) Dilute **HDAC6 human recombinant enzyme** in **HDAC Assay Buffer** to 7 ng/µl (35 ng/reaction)*. Aliquot any remaining enzyme and store undiluted at -80℃. 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.*

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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 **Fluorogenic 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 in DMSO (20 µM)** to the well 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 **HDAC6 human recombinant enzyme** to the wells designated "Positive Control," "Test Inhibitor," and "Inhibitor Control." Incubate at 37 ºC for 30 min.

	"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 (20 µM)	–	–	–	5 µl
Test Inhibitor	–	–	5 µl	–
Inhibitor buffer (no inhibitor)	5 µl	5 µl	–	
Diluted HDAC6 (7 ng/µl)	–	5 µl	5 µl	5 µl
Total	50 µl	50 µl	50 µl	50 µl

Step 2:
Add 50 µl of undiluted **2x HDAC Developer** 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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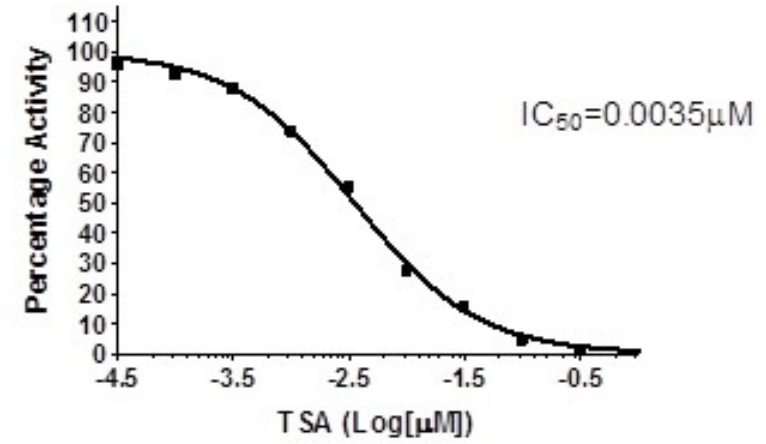
Example of Assay Results:



HDAC6 enzyme activity, measured using the *HDAC6 Chemiluminescent Assay Kit*, AMS Bio Catalog #AMS.50076. Fluorescence was measured using a Bio-Tek fluorescent microplate reader.

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