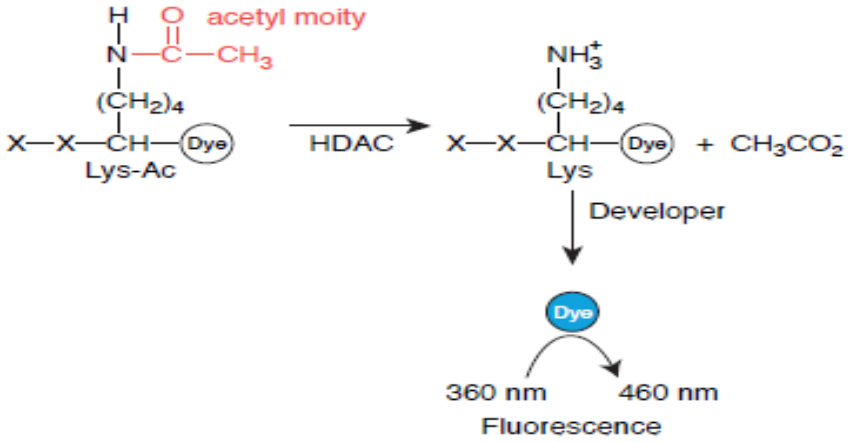
Data Sheet

Fluorogenic HDAC Assay Kit

Catalog #: AMS.50033
Size: 96 Reactions

DESCRIPTION: The *Fluorogenic HDAC Assay Kit* is a complete assay system designed to measure histone deacetylase (HDAC) class 1 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 HDAC2 enzyme and a potent HDAC inhibitor, Trichostatin A, for use as a positive and negative control. The *Fluorogenic HDAC 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.



COMPONENTS:

Catalog #	Component	Amount	Storage	
AMS.50002	HDAC2 human recombinant enzyme	2 µg	-80°C	***Avoid freeze/ thaw cycles!***
AMS.50037	Fluorogenic HDAC substrate 3 (5 mM)	50 µl	-80°C	
AMS.50030	2x HDAC Developer (contains Trichostatin A) (50 µM)	6 ml	-80°C	
	Trichostatin A (200 µM)	100 µl	-20°C	
AMS.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:
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

APPLICATIONS: Great for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications. This kit is suitable for class 1 and class 2b HDACs (HDACs 1, 2, 3, 6, 10). For class 2a and class 4 HDACs (HDACs 4, 5, 7, 9, 11), we recommend the Fluorogenic HDAC Class 2a Assay Kit, Cat. #AMS.50041, or for HDAC8, we recommend the Fluorogenic HDAC8 Assay Kit, Cat. #AMS.50068.

STABILITY: One year from date of receipt when stored as directed.

REFERENCE(S):

1. A. Ito *et al.* (2001) *EMBO J.* **20** 1331.
2. N.A. Barlev *et al.* (2001) *Mol. Cell* **8** 1243.
3. A. Ito *et al.* (2002) *EMBO J.* **21** 6236.

ASSAY PROTOCOL:

Immediately prior to assay:

1) Dilute **Trichostatin** A (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 200 µM **Trichostatin A** stock solution in aliquots at -80°C.)

2) Dilute **Fluorogenic HDAC substrate 3 (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 **HDAC2** in **HDAC assay buffer** to 1 ng/µl (5ng/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 **Fluorogenic HDAC substrate 3 (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** (20 µM) to the well designated "Inhibitor Control". *Keep final DMSO concentration at or below 1%.*

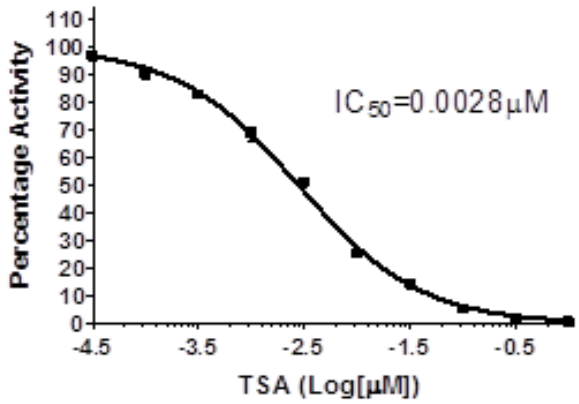
3) Add 5 µl of **HDAC Assay Buffer** to the wells designated "Blank".

4) Initiate reaction by adding 5 µl of diluted **HDAC2** enzyme to the wells designated “Positive Control”, "Test Inhibitor", and "Inhibitor Control". Incubate at 37ºC 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 HDAC2 (1 ng/µl)	–	5 µl	5 µl	5 µl
Total	50 µl	50 µl	50 µl	50 l

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:



HDAC2 enzyme activity, measured using the *HDAC2 Fluorogenic Assay Kit*, Catalog #50033. Fluorescence was measured using a Bio-Tek fluorescent microplate reader. *Data shown is lot-specific.*

RELATED PRODUCTS:

Product Name	Catalog #	Size
HDAC2 (C-His)	AMS.50002	50 µg
HDAC2 (C-Flag)	AMS.50052	50 µg
HDAC1	AMS.50051	50 µg
HDAC3/NcoR2	AMS.50003	50 µg
HDAC4	AMS.50004	10 µg
HDAC5	AMS.50005	10 µg
HDAC6 (C-Flag)	AMS.50056	50 µg
HDAC6 (N-GST)	AMS.50006	50 µg
HDAC6 (H216A)	AMS.50046	50 µg
HDAC6 (H611A)	AMS.50066	50 µg
HDAC7	AMS.50007	10 µg
HDAC8	AMS.50008	50 µg
HDAC9	AMS.50009	10 µg
HDAC10	AMS.50010	50 µg
HDAC11	AMS.50011	50 µg
HDAC2 Assay Kit	AMS.50062	96 reactions
HDAC Assay Kit (Green)	AMS.50034	96 reactions
HDAC Class 2a Assay Kit	AMS.50041	96 reactions
HDAC1 Assay Kit	AMS.50061	96 reactions
HDAC3 Assay Kit	AMS.50073	96 reactions
HDAC6 Assay Kit	AMS.50076	96 reactions
HDAC8 Assay Kit	AMS.50068	96 reactions

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