Amplite™ Fluorimetric HDAC Activity Assay Kit

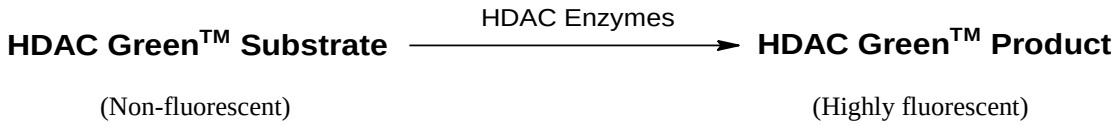
Green Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 13601 (200 assays)	Keep in freezer and avoid exposure to light	Fluorescence microplate readers

Introduction

Histone deacetylases (HDAC) are a class of enzymes that remove acetyl groups from a ε-N-acetyl lysine amino acid on a histone. Deacetylation restores the positive electric charge of the lysine amino acids, which increases the histone's affinity to the negatively charged phosphate backbone of DNA. This process generally down-regulates DNA transcription by blocking the access of transcription factors. HDAC inhibitors are being studied as a treatment for cancer.

Our Amplite™ Fluorimetric HDAC Activity Assay Kit provides a quick, convenient, and sensitive method for the detection of HDAC activity. This kit uses our non-peptide HDAC Green™ substrate that is much more sensitive than the peptide-based HDAC substrates such as Ac-RGK(Ac)-R110, Ac-RGK(Ac)-AMC and Ac-RGK(Ac)-AFC. In addition, HDAC Green™ substrate is also much more resistant to protease hydrolysis than other commercial peptide-based HDAC substrates. Our kit can be used for measuring HDAC activity in cell lysates or HDAC inhibitor screening with cell extracts or purified enzymes. The long wavelength emission of the HDAC Green™ substrate makes the assay less interfered from compounds and cell components. HDAC activity is monitored with excitation at 490 nm and emission at 525 nm.



Kit Key Features

Broad Application:	Can be used for quantifying HDAC in solutions and in cell extracts.
Continuous:	Easily adapted to automation without a separation step.
Convenient:	Formulated to have minimal hands-on time. No wash is required.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Components	Amount
Component A: HDAC Green™ Substrate	1 vial (40 µL)
Component B: Assay Buffer	1 bottle (40 mL)
Component C: HDAC Inhibitor (Trichostain A, 3 mM)	1 vial (20 µL)
Component D: Signal Enhancer (50X)	1 vial (200 µL)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare HDAC containing samples (40 µL) → Add HDAC inhibitor or test compounds (10 µL) → Incubate at room temperature or 37 °C for 10-20 minutes → Add HDAC Green™ Substrate working solution (50 µL) → incubate at room temperature or 37 °C for 30 -60 minutes → Monitor fluorescence intensity at Ex/Em = 490/525 nm

Note: Thaw all the kit components before starting the experiment.

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

1. Prepare working solution:

1.1 Prepare HDAC-containing test samples: Dilute 5–10 mg/mL of HeLa nuclear extract or cell lysates at 1:40 in Assay Buffer (Component B).
Note: 40 µL of the diluted sample is enough for one well of a 96-well plate. Dilute extract immediately before use. Store the solution on ice.

1.2 Prepare dilutions of HDAC inhibitor (Trichostain A) solution: Dilute 3 mM Trichostatin A solution (Component C) at 1:100 in assay buffer (Component B) to get a 30 µM Trichostatin A solution. Add 10 µL of the 30 µM Trichostatin A solution into each inhibitor control well.

1.3 Prepare HDAC Green™ Substrate working solution: Add 20 µL of HDAC Green™ Substrate (Component A) and 100 µL of the Signal Enhancer (Component D) into 5 mL of Assay Buffer (Component B).
Note1: The diluted HDAC Green™ Substrate working solution is not stable, 5 mL of the diluted HDAC Green™ Substrate working solution is enough for 100 assays.
Note2: Prepare fresh HDAC Green™ Substrate working solution for each experiment. Keep reconstituted working solution on ice until use.

2. Run HDAC Assay:

2.1 Add 40 µL of diluted nuclear extract, enzyme solution or other HDAC samples and 10 µL of test compounds to the corresponding microplate wells (see Table 1).
For positive control: Add 40 µL of diluted HDAC enzyme solution or HeLa nuclear extract (from Step 1.1) with 10 µL of Assay Buffer (Component B).
For negative control: Add 40 µL of diluted HeLa nuclear extract (from Step 1.1) with 10 µL of 30 µM Trichostatin A solution (from Step 1.2), or use a known sample containing no HDAC activity.
For Blank (no Enzyme): Add 50 µL of Assay Buffer (Component B) only.

2.2 Incubate the plate at room temperature or 37 °C for 10 - 20 minutes.
Note: For screening HDAC inhibitor, preincubate the compounds with HeLa nuclear extract or pure enzyme before adding HDAC Green™ Substrate working solution (see Step 2.3)

2.3 Add 50 µL of HDAC Green™ Substrate working solution (from Step 1.3) into each well. Incubate the plate at room temperature or 37 °C for 30-60 minutes.

2.4 Monitor fluorescence intensity at Ex/Em = 490/525 nm.

Table 1. Layout of nuclear extracts with test compounds in a solid black 96-well microplate

Samples	HeLa Extract (from Step 1.2)	Assay Buffer (Component B)	Trichostatin A (from Step 1.3)	Test Compounds	HDAC Green™ Substrate (from Step 1.1)
Blank (no Enzyme)	0 µL	50 µL	0 µL	0 µL	50 µL
Positive Control	40 µL	10 µL	0 µL	0 µL	50 µL
Negative Control	40 µL	0 µL	10 µL	0 µL	50 µL
Test Compounds	40 µL	0 µL	0 µL	10 µL	50 µL

Data Analysis

The fluorescence in blank wells (with the assay buffer only) is used as the background fluorescence, and is subtracted from the values for those wells with the HDAC Green™ reactions. All fluorescence readings are expressed in relative fluorescence units (RFU). The typical data are shown in Figure 1.

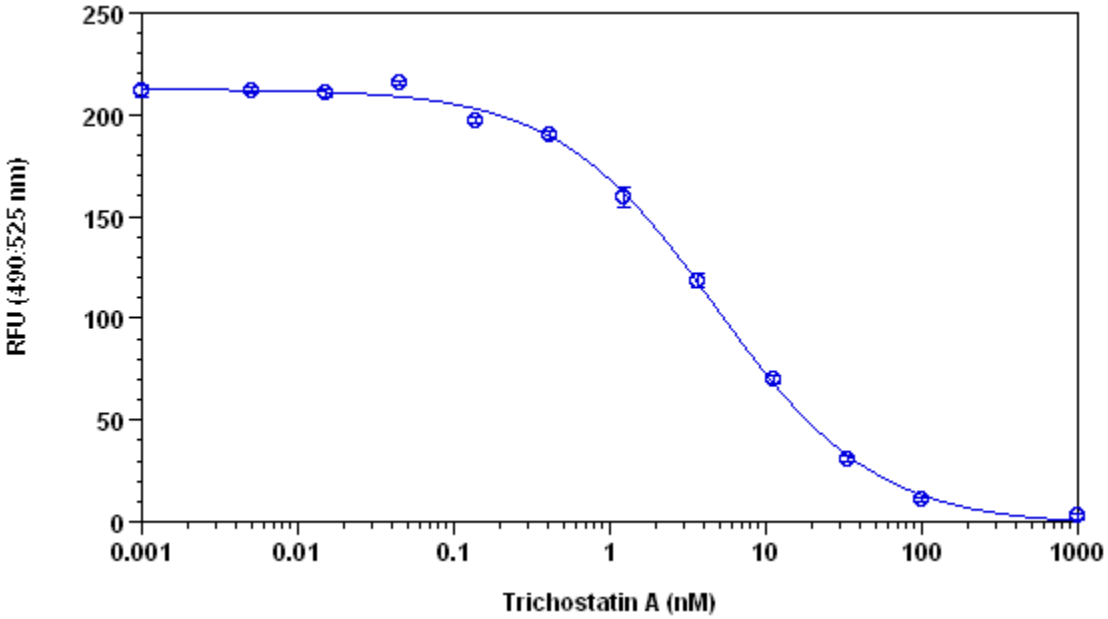


Figure 1. Trichostatin A inhibition in HeLa nuclear extract was measured with Amplite™ Fluorimetric HDAC Activity Assay Kit using Gemini Fluorescence microplate reader (Molecular Devices).

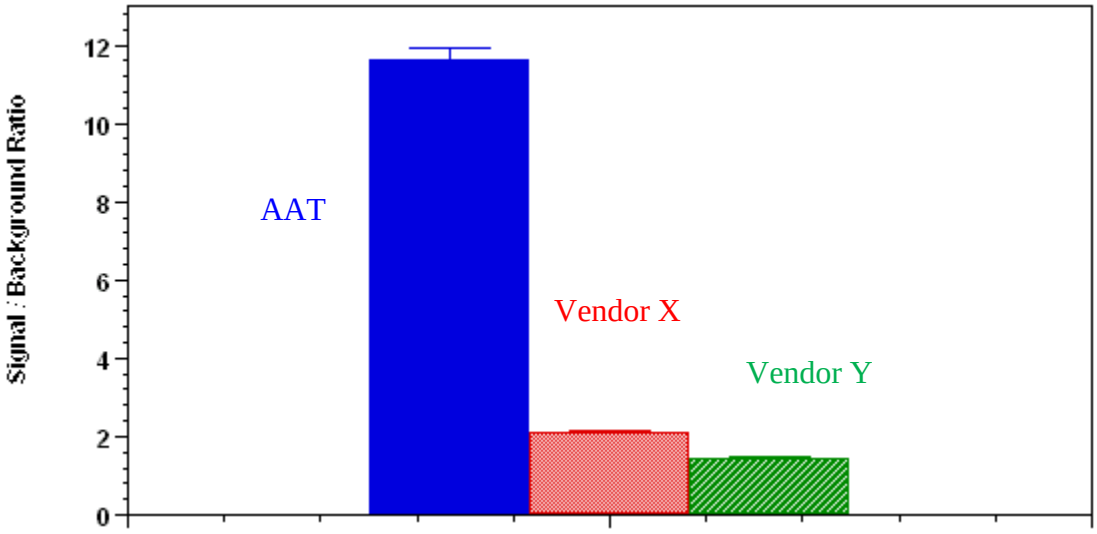


Figure 2. HDAC activity in HeLa nuclear extract was measured with Amplite™ Fluorimetric HDAC Activity Assay Kit (in blue) was compared with Vendor X (in red) and Vendor Y (in green), both of which use Ac-RGK(Ac)-R110 peptide substrate. The signal/background ratio of the HDAC activity measured with Amplite™ Fluorimetric HDAC Activity Assay Kit is more than10 times higher than those of Vendors X and Y.

References

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