AAT Bioquest®

PRODUCT INFORMATION SHEET

Amplite™ Fluorimetric HDAC Activity Assay Kit *Green Fluorescence*

Catalog number: 13601
Unit size: 200 Tests

Component	Storage	Amount
Component A: HDAC Green™ Substrate	Freeze (< -15 °C), Minimize light exposure	1 vial (40 µL)
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (40 mL)
Component C: HDAC Inhibitor (Trichostain A, 3 mM)	Freeze (< -15 °C), Minimize light exposure	1 vial (20 µL)
Component D: Signal Enhancer (50X)	Freeze (< -15 °C), Minimize light exposure	1 vial (200 µL)

OVERVIEW

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 for the negatively charged phosphate backbone of DNA. This generally down-regulates DNA transcription by blocking the access of transcription factors. HDACs are involved in the pathway by which the retinoblastoma protein (pRb) suppresses cell proliferation. The pRb protein is part of a complex which attracts HDACs to the chromatin so that it will deacetylate histones. HDAC inhibitors are being studied as a treatment for cancer. The Amplite™ HDAC Assay Kit provides a quick, convenient, and sensitive method for the detection of HDAC activity. Our HDAC Green™ substrate is a non-peptide compound that is much more resistant than other commercial peptide-based HDAC substrates. Our kit can be used for measuring HDAC activity in cell lysates, in vitro inhibitor screening with extracts or purified enzymes. The long wavelength emission and higher extinction coefficient of the HDAC Green™ substrate provide less interference from compounds and cell components. HDAC activity is determined by monitoring the green fluorescence enhancement with excitation at 490 nm and emission at 520 nm.

AT A GLANCE

Protocol Summary

1. Prepare HDAC containing samples (40 µL)
2. Add HDAC inhibitor or test compounds (10 µL)
3. Incubate at room temperature or 37°C for 10 - 20 minutes
4. Add HDAC Green™ Substrate working solution (50 µL)
5. Incubate at room temperature or 37°C for 30 - 60 minutes
6. Monitor fluorescence intensity at Ex/Em = 490/525 nm (Cutoff = 515 nm)

Important Thaw all the kit components before starting the experiment.

KEY PARAMETERS

Fluorescence microplate reader

Excitation	490 nm
Emission	525 nm
Cutoff	515 nm
Recommended plate	Solid black

PREPARATION OF WORKING SOLUTION

1. 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.

2. HDAC inhibitor (Trichostain A) solution (30 µM)
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.

3. 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).

Note 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. Prepare fresh HDAC Green™ Substrate working solution for each experiment. Keep reconstituted working solution on ice until use.

SAMPLE EXPERIMENTAL PROTOCOL

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

Samples	HeLa Extract	Assay Buffer (Component B)	Trichostatin A (HDAC inhibitor)	Test Compounds	HDAC Green™ Substrate
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 Compound	40 µL	0 µL	0 µL	10 µL	50 µL

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.

   a. For positive control: Add 40 µL of diluted HDAC enzyme solution or HeLa nuclear extract with 10 µL of Assay Buffer (Component B).

   b. For negative control: Add 40 µL of diluted HeLa nuclear extract with 10 µL of 30 µM Trichostatin A solution, or use a known sample containing no HDAC activity.

   c. For Blank (no Enzyme): Add 50 µL of Assay Buffer (Component B) only.

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.

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

4. Monitor fluorescence intensity at Ex/Em = 490/525 nm. (Cutoff = 515 nm)

EXAMPLE DATA ANALYSIS AND FIGURES

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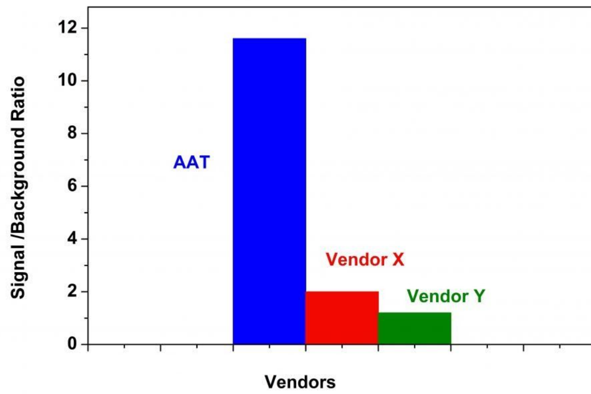

Figure 1. HDAC activity in HeLa nuclear extract was measured with Amplite™ Fluorimetric HDAC Activity Assay Kit (in blue) was compared with Vendor X (in red) and Vendor Y (in green), both of which use Ac-RGK(Ac)-R110 peptide substrate. The signal/background ratio of the HDAC activity measured with Amplite™ Fluorimetric HDAC Activity Assay Kit is more than 10 times higher than those of Vendors X and Y.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.

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