CREATIVE BIOMART®
Assay Kit

Fluorimetric HDAC Activity Assay Kit (Green)

Product Information

Cat

Kit-0983

Common Name

HDAC

Cat.No. Kit-0983

Product Overview

Our 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.

Description

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.

Storage

Keep in freezer and avoid exposure to light.

Size

200 assays

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Kit Components

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

Features & Benefits

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.

Preparation

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 uL 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 uL of HDAC Green Substrate (Component A) and 100 uL 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.

Assay Protocol

2. Run HDAC Assay:

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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 (from Step 1.1)	HDAC Green Substrate
Blank (no Enzyme)	0 uL	50 uL	0 uL	0 uL	50 uL
Positive Control	40 uL	10 uL	0 uL	0 uL	50 uL
Negative Control	40 uL	0 uL	10 uL	0 uL	50 uL
Test Compounds	40 uL	0 uL	0 uL	10 uL	50 uL

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).