

Elite™ HDAC Activity Assay Kit (Green Fluorescence)

CATALOG NUMBER: CA-H100, 200 assays

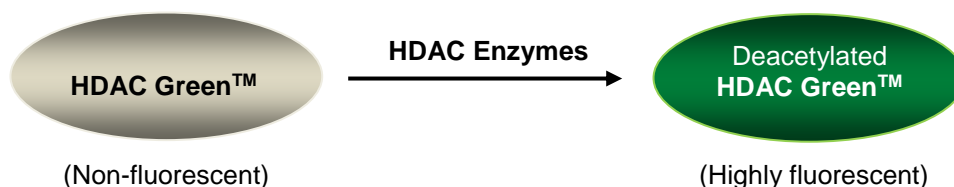
Introduction

HDAC (Histone deacetylases), are a class of enzymes that remove acetyl groups from an ϵ -N-acetyl lysine amino acid on a histone. HDAC inhibitors have been shown to induce specific changes in gene expression and to influence a variety of other processes, including growth arrest, differentiation, cytotoxicity and induction of apoptosis.

Histone deacetylase (HDAC) inhibitors have an unprecedented potential to occupy a major position in the future market of anticancer agents. However, progress in the development of these new chemotherapeutics is largely dependent on the existence of bioassays well-suited for inhibitor screening. Herein, we summarize recent developments in HDAC assay technology and, particularly, discuss different assay types with respect to their suitability for high-throughput screening programs.

Product Description

Elite™ 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.



Features

- **Broad application:** could measure the HDAC activities in solution or 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 requirement for waste disposal.

Kit Components

- | | |
|---|----------------------|
| • Component A: (HDAC Green™ Substrate) | 1 vial (40 μ l) |
| • Component B: (Assay Buffer) | 1 bottle (40 ml) |
| • Component C: (HDAC Inhibitor, 3mM Trichostain A) | 1 vial (20 μ l) |
| • Component D: (50x Signal Enhancer) | 1 vial (200 μ l) |

Storage

Keep in freezer (-20 °C) and avoid exposure to light.



Assay Protocol (for 96-Well Plate)

1. Working solution preparation:

- 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 the extract immediately before use. Store the solution on ice.

- 1.2 Prepare dilutions of HDAC inhibitor (Trichostatin 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. HDAC activity 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.

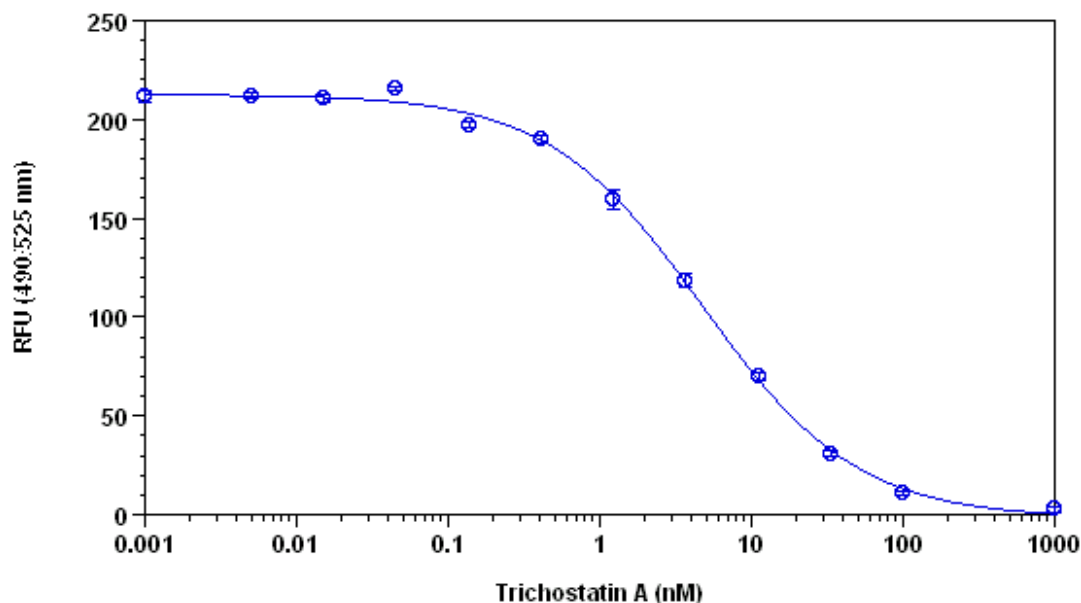


Figure 1. Trichostatin A inhibition in HeLa nuclear extract was measured with Elite™ HDAC Assay Kit using Gemini Fluorescence Microplate Reader (Molecular Devices).

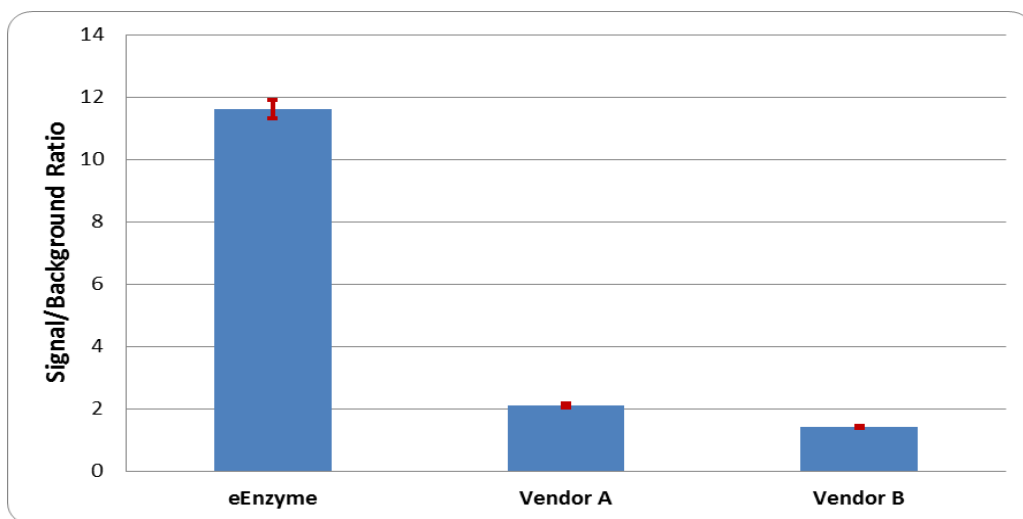


Figure 2. HDAC activity in HeLa nuclear extract was measured with Elite™ HDAC Activity Assay Kit was compared with similar assay kit in the market by Vendor A and B, both of which use Ac-RGK(Ac)-R110 peptide substrate. The signal/background ratio of the HDAC activity measured with Elite™ Fluorimetric HDAC Activity Assay Kit is more than 10 times higher than those of Vendors A and B.



References

1. Belien A, De Schepper S, Floren W, Janssens B, Marien A, King P, Van Dun J, Andries L, Voeten J, Bijmens L, Janicot M, Arts J. (2006) Real-time gene expression analysis in human xenografts for evaluation of histone deacetylase inhibitors. *Mol Cancer Ther*, 5, 2317.
2. Takahashi-Fujigasaki J, Fujigasaki H. (2006) Histone deacetylase (HDAC) 4 involvement in both Lewy and Marinesco bodies. *Neuropathol Appl Neurobiol*, 32, 562.
3. Olaharski AJ, Ji Z, Woo JY, Lim S, Hubbard AE, Zhang L, Smith MT. (2006) The histone deacetylase inhibitor trichostatin a has genotoxic effects in human lymphoblasts in vitro. *Toxicol Sci*, 93, 341.
4. Voelter-Mahlknecht S, Ho AD, Mahlnecht U. (2005) FISH-mapping and genomic organization of the NADdependent histone deacetylase gene, Sirtuin 2 (Sirt2). *Int J Oncol*, 27, 1187.
5. Klampfer L, Huang J, Swaby LA, Augenlicht L. (2004) Requirement of histone deacetylase activity for signaling by STAT1. *J Biol Chem*, 279, 30358.
6. Nusinzon I, Horvath CM. (2003) Interferon-stimulated transcription and innate antiviral immunity require deacetylase activity and histone deacetylase 1. *Proc Natl Acad Sci USA*, 100, 14742.

