

Product Information

Histone Deacetylase Assay Kit, Fluorometric

Catalog Number **CS1010**
Storage Temperature $-20\text{ }^{\circ}\text{C}$

TECHNICAL BULLETIN

Synonym: HDAC Assay Kit

Product Description

Histone deacetylases (HDAC) are enzymes that remove acetyl groups from histone proteins. HDAC are important regulators of gene expression and genome function. They are implicated in a number of human disease states, namely several cancers, neurological disorders, and aging. Therefore, HDAC are relevant key targets for therapeutic intervention. Moreover, histone deacetylase inhibitors have been shown to serve as anti-tumor agents.¹⁻²

The Histone Deacetylase Assay Kit provides a simple method for the detection of HDAC activity based on a two-step enzymatic reaction. The substrate for this enzymatic assay is a substituted peptide with an acetylated lysine residue and a bound fluorescent group. The first step of the reaction is deacetylation of the acetylated lysine side chain by the HDAC containing sample (HeLa cell extract, purified enzyme, etc.). The second step is the cleavage of the deacetylated substrate by the Developer Solution and the release of the free highly fluorescent group. The measured fluorescence is directly proportional to the deacetylation activity of the sample.³⁻⁵

The kit includes all the reagents required for the fast and easy measurement of HDAC activity in cell or nuclear extracts, or with purified enzyme preparations. In addition, the kit provides HeLa Cell Lysate as a source for HDAC activity for inhibitor screening or as a positive control, a HDAC inhibitor (Trichostatin A), and a standard to enable activity quantitation.

The Histone Deacetylase Assay Kit has been tested on HeLa, HEK 293T, NIH 3T3, and U 937 cell extracts.

Components

The kit is sufficient for 100 assays in 96 well plates

Assay Buffer Catalog Number A6480	20 ml
Developer Solution Catalog Number D5068	1.5 ml
HDAC Inhibitor (Trichostatin A), 1 mM Catalog Number H6664	50 μl
HDAC Substrate, 20 mM Catalog Number H6539	50 μl
HeLa Cell Lysate Catalog Number H6414	500 μl
Standard (Non-acetylated substrate), 4 mM Catalog Number S7196	50 μl

Equipment Needed but Not Provided

- 96 well plates, Nunc® FluoroNunc™, black (Catalog Number P8741)
- Fluorimeter

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

HDAC Substrate Solution - Thaw the HDAC Substrate (Catalog Number H6539) at room temperature and mix until homogenous. Dilute an aliquot of the HDAC Substrate 100-fold with Assay Buffer (Catalog Number A6480). Store the remaining HDAC Substrate (Catalog Number H6539) at $-20\text{ }^{\circ}\text{C}$. 50 μl of the diluted HDAC Substrate Solution are sufficient for a single reaction (a single well).

HDAC Inhibitor Solution - Thaw the HDAC Inhibitor (Catalog Number H6664) at room temperature and mix until homogenous. Dilute an aliquot of the HDAC Inhibitor 40-fold with Assay Buffer (Catalog Number A6480). Store the remaining HDAC Inhibitor (Catalog Number H6664) at $-20\text{ }^{\circ}\text{C}$. 5 μl of the diluted inhibitor is sufficient for a single reaction (a single well).

Standard Solution - Thaw the Standard (Non-acetylated substrate, Catalog Number S7196) at room temperature and mix until homogenous. Dilute an aliquot of the Standard 200-fold with Assay Buffer (Catalog Number A6480). Store the remaining Standard at $-20\text{ }^{\circ}\text{C}$. 100 μl of the diluted Standard Solution is sufficient for a single reaction (a single well).

Storage/Stability

The kit is shipped on dry ice and storage at $-20\text{ }^{\circ}\text{C}$ is recommended. After the first thaw, freeze the HeLa Cell Lysate (Catalog Number H6414) in working aliquots at $-70\text{ }^{\circ}\text{C}$ to avoid loss of activity.

Procedure

The assay for HDAC activity and determination of the standard curve are described as separate procedures. If required, both procedures can be performed in parallel on the same plate. In this case the standard samples can be incubated with the reaction samples, before the addition of the developer.

Thaw the kit components and mix until homogenous. Keep the HeLa Cell Lysate and the Developer Solution on ice until used.

Set the wavelengths of fluorimeter plate reader:
excitation wavelength of 350-380 nm
emission wavelength of 440-480 nm

Optimize the wavelengths according to the instrument used.

Assay for HDAC activity or inhibitor screening

Perform the reactions in duplicates.

1. Add the reaction reagents to the wells of a 96 well plate according to Table 1.

Table 1.

Reaction Scheme for HDAC Activity or Inhibitor Screening

Component	Assay Buffer (μl)	HDAC Inhibitor Solution (μl)	HeLa Lysate or Test Sample (μl)*	HDAC Substrate Solution (μl)
HDAC Activity Assay	50-x	-	x (≤ 20)	50
HDAC Activity Inhibition Assay	45-x	5	x (≤ 20)	50
Blank	50	-	-	50

* For HeLa Cell Lysate use 5–15 μl

2. Incubate the plate at $30\text{ }^{\circ}\text{C}$ for 30 minutes (or longer if desired).
3. Add 10 μl of Developer Solution (Catalog Number D5068) to each well.
4. Incubate the plate at room temperature for 10 minutes.
5. Measure the fluorescence with the fluorimeter plate reader.

Determination of the Standard Curve

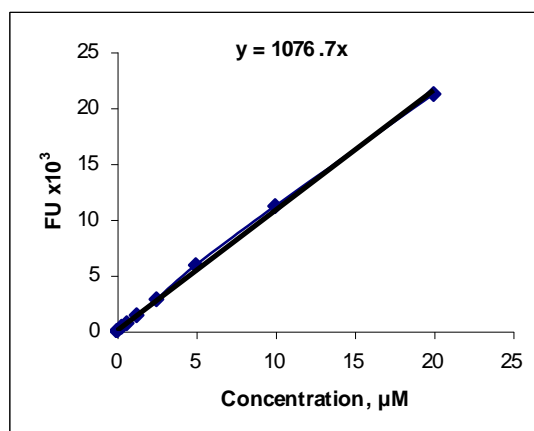
The standard curve, as described, should be performed in two sets. The first set is a series to measure the fluorescence of different concentrations of the standard and the second set without developer is a standard blank.

1. For each set, leave the first well empty. Add 100 μl of the Assay Buffer to the next 7 wells of each set (i.e., add the buffer to wells 2-8).
Note: steps 2-5 should be performed for both sets.
2. Add 100 μl of the 200-fold diluted Standard Solution to the first and second well and mix thoroughly.
3. Transfer 100 μl of the mixture from the second well to the third well containing 100 μl of Assay Buffer and mix well.
4. Continue the sequential dilution by transferring 100 μl of solution (out of 200 μl) from well to well, as detailed in the previous step ending up at the seventh well (i.e., ending with seven standard concentrations). Discard 100 μl from the last dilution (the seventh well). The eighth (last) well contains 100 μl of the Assay Buffer to be used as the blank.
5. Add 10 μl of Developer Solution to each well in one set of the standard dilution series. To the second set, add 10 μl of Assay Buffer instead of Developer Solution to each well.
6. Incubate the plate at room temperature for 10 minutes.
7. Read the plate in the plate reader.
8. Plot the fluorescence signal (y-axis) versus concentration of the Standard (x-axis). Determine the slope as FU/ μM .

Results

Calculate the HDAC concentration from the standard curve.

Figure 1.
Typical Standard Curve



References

1. Monneret, C., Histone deacetylase inhibitors. *Eur. J. Med. Chem.*, **40**, 1-13 (2005).
2. Vigushin, D.M. *et al.*, Trichostatin A is a histone deacetylase inhibitor with potent antitumor activity against breast cancer *in vivo*. *Clin. Cancer Res.*, **7**, 971-976 (2001).
3. Heltweg, B., and Jung, M., A homogeneous nonisotopic histone deacetylase activity assay. *J. Biomol. Screen.*, **8**, 89-95 (2003).
4. Hoffmann, K. *et al.*, First non-radioactive assay for *in vitro* screening of histone deacetylase inhibitors. *Pharmazie*, **55**, 601-606 (2000).
5. Wegener, D. *et al.*, A fluorogenic histone deacetylase assay well suited for high-throughput activity screening. *Chem. Biol.*, **10**, 61-68 (2003).

Under license from CycLex Co. Ltd. Japan, Patent No EP 1243658 Applications No WO140506, US200382668, JP2001149081, CA2392711

Nunc is a registered trademark of Nunc A/S Corporation.
FluoroNunc is a trademark of Nunc A/S Corporation.

MK, KAA, EB, MAM 12/18-1