Introduction and Background

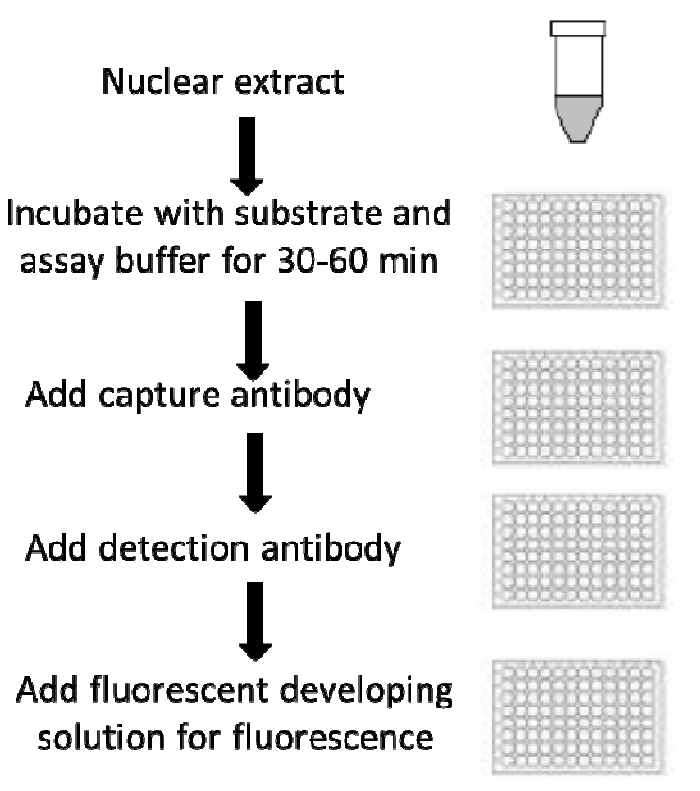
A. Overview

Histone deacetylases (HDACs) play a critical role in transcriptional repression of the gene expression in eukaryotic cells through catalyzing the hydrolytic removal of acetyl groups from histone lysine residues. HDACs are tightly involved in cell cycle regulation, cell proliferation, and in development of human cancer. HDAC inhibition displays significant effects on apoptosis, cell cycle arrest, and differentiation in cancer cells. HDAC inhibitors are currently being developed as potential anticancer agents. There are several methods used for measuring HDAC activity/ inhibition. However most of these methods available so far are time consuming, laborious, produce radioactive waste, or cannot measure precise HDAC activity and inhibitory effects of inhibitors. The HDAC Activity/Inhibition Assay Kit uses a proprietary and unique procedure to measure HDAC activity/inhibition with the following features:

- Fast procedure, which can be finished within 3 hours.
- Innovative colorimetric assay without need for radioactivity, extraction, and chromatography.
- Direct measurement of HDAC activity and inhibition with no use of lysyl endopeptidase, thereby avoiding the false inhibitory effect on HDACs and allowing more accurate measurement.
- Strip microplate format makes the assay flexible: manual or high throughput analysis.
- Simple, reliable, and consistent assay conditions.

B. Test Principle

The HDAC Activity/Inhibition Assay Kit is designed for measuring total HDAC activity/inhibition. In an assay with this kit, the unique acetylated histone substrate is stably captured on the strip wells. Active HDACs bind to and deacetylate histone substrate. The remaining un-deacetylated substrate can be recognized with high affinity acetylated histone antibody. The ratio or amount of the un-deacetylated histone, which is inversely proportional to HDAC enzyme activity, can be then colorimetrically quantified through an ELISA-like reaction.



C. Notice for Application of Kit

✓ This kit has been configured for research use only and is not for diagnostic and clinical use.

D. Application

For measuring HDAC activity/inhibition from a broad range of species including mammalian cells/tissues, plants, and bacteria.

Material and Method

A. List of component

	96 assays
H1 (10X wash buffer)	**28 ml**
H2 (HDAC assay buffer)	**3 ml**
H3 (biotinylated HDAC substrate)*	**100 µl**
H4 (HDAC inhibitor, 0.5 mM)*	**100 µl**
H5 (HDAC standard, 20 µg/ml)*	**50 µl**
H6 (capture antibody 1000 µg/ml)*	**50 µl**
H7 (detection antibody 200 µg/ml)*	**20 µl**
H8 (developing solution)	**12 ml**
H9 (stop solution)	**6 ml**
8 well assay strip (with frame)	**12**

* For maximum recovery of the products, centrifuge the original vial after thawing prior to opening the cap.

B. Additional Required Materials But Not Provided

1. Orbital shaker
2. Pipettes and pipette tips
3. Microplate reader
4. 1.5 ml microcentrifuge tubes

C. Stability and storage

Upon receipt, store H3, H4, H5 and H7 at –20℃ away from light. Store H6, H8 and 8 well assay strips at 4℃ away from light. Store all other components at room temperature. The components of the kit should be stable for 6 months when stored properly.

D. Protocol

1. Prepare nuclear extracts by using you own successful method. Nuclear extracts can be used immediately or stored at -80℃ for future use.
2. Determine number of the strip wells required. Leave these strips in the plate frame (remaining unused strips can be put back in the bag. Seal the bag tightly and store at 4℃). Dilute 10 X H1 with distilled water to 1 X H1 (pH 7.2-7.5).
3. Dilute H3 at the 1:50 ratios with 1 X H1 and add 50 µl of the diluted H3 into each well except the wells for blank and standard curve. For the blank, add 50 µl of 1 X H1 into the wells (no H3 added). For preparation of standard curve, add 50 µl of 1 X H1 into the wells (no H3 added) followed by adding 1 µl of H5 at different amount (0.1-10 ng). Cover the wells with Parafilm M and incubate at room temperature for

30-45 min.

4. Aspirate and wash each well with 150 µl of 1 X H1 two times.
5. Add 28 µl of H2, and 2 µl of nuclear extracts (4-20 µg) or HDAC enzymes to the strip wells except the wells for the control, blank and standard curve. For the control and standard curve, instead of nuclear extract add 2 µl of H2. For HDAC inhibition, add 2 µl of different amounts of H4 or tested inhibitors and reduce H2 volume to 26 µl. For blank, add 30 µl of H2 into the wells. Mix, cover the strip wells and incubate at 370C for 45-60 min.
6. Aspirate and wash each well with 150 µl of 1 X H1 three times.
7. Dilute H6 (at the 1:1000 ratios) to 1 µg/ml with 1 X H1. Add 50 µl of the diluted H6 to each strip well and incubate at room temperature for 60 min on an orbital shaker (50-100 rpm).
8. Aspirate and wash each well with 150 µl of 1 X H1 for 4 times.
9. Dilute H7 (at the 1:1000 ratios) to 0.2 µg/ml with 1 X H1. Add 50 µl of the diluted H7 to each strip well and incubate at room temperature for 25-30 min.
10. Aspirate and wash each well with 150 µl of 1 X H1 for 4-5 times.
11. Add 100 µl of H8 to each well and incubate at room temperature for 2-10 min away from light. Monitor color development in the sample and standard well (blue).
12. Add 50 µl of H9 to each well to stop enzyme reaction when color in the standard wells containing the higher concentrations of standard control turns medium blue. The color should change to yellow and absorbance can be read on a microplate reader at 450 nm within 2-15 min.
13. Calculate HDAC activity or inhibition. For simple calculation:

$$\text{HDAC activity (OD/h/ml)} = \frac{[\text{OD (control} - \text{blank)} - \text{OD (sample} - \text{blank)}]}{\text{reaction time (0.5-1 h)}} \times \text{sample dilution*}$$

$$\text{HDAC activity (OD/h/ml)} = \frac{[\text{OD (control} - \text{blank)} - \text{OD (sample} - \text{blank)}]}{\text{reaction time (0.5-1 h)}} \times \text{sample dilution*}$$

For an accurate calculation, plot Delta RFU value versus amount of H5 and determine the slope as delta RFU/ng.

Calculate HDAC activity using the following formula:

$$\text{Activity (ng/h/ml)} = \frac{[\text{OD (control-blank)} - \text{OD (sample} - \text{blank)}]}{\text{Slope x reaction time (0.5-1 h)}} \times \text{sample dilution*}$$

Troubleshooting

- **No Signal for Only the Sample**

The protein sample is not properly extracted.	Ensure the nuclear protein extraction protocol is suitable for nuclear protein extraction.
The protein amount is added into well insufficiently.	Ensure extract contains enough amount of proteins.
The sample is not prepared from fresh cells or tissues.	The nuclear extracts from frozen cells or tissues significantly loss enzyme activity. The fresh sample should be used.
Nuclear extracts are incorrectly stored.	Ensure the nuclear extracts are stored at –80°C.
Reagents are added incorrectly.	Check if reagents are added in order and if some steps of the procedure are omitted by mistake.
Incubation time and temperature is incorrect.	Ensure the incubation time and temperature described in the protocol are correctly followed.
Absence of HDAC activity in the sample due to treatment.	N/A

- **High Background Present for the Blank**

The well is not washed enough.	Check if wash at each step is performed according to the protocol.
Overdevelopment.	Decrease development time in Step 11.