Introduction and Background

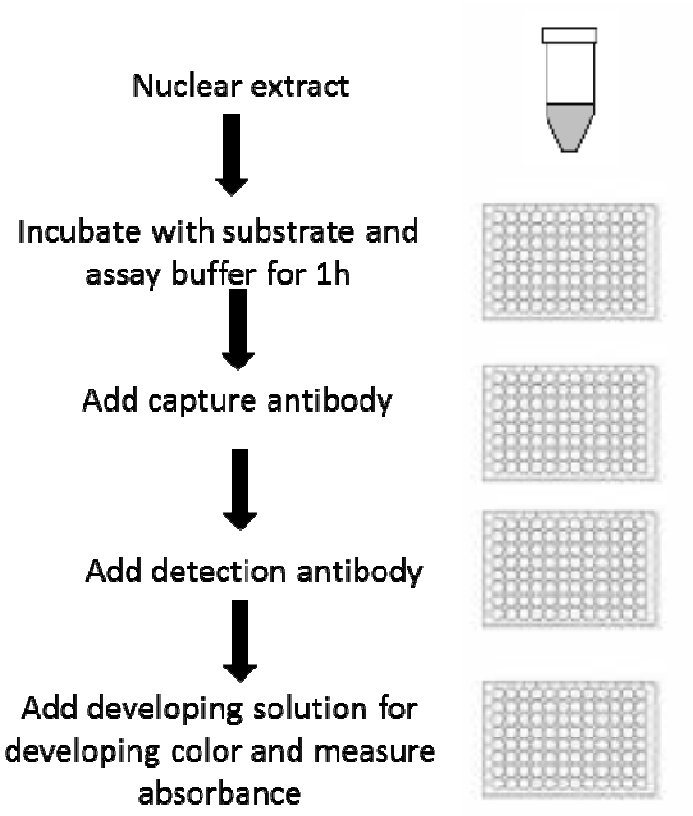
A. Overview

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. Three distinct families of HDACs have been described, comprising a group of at least 20 proteins in humans. HDAC1 is a class I histone deacetylase containing 482 amino acid residues. HDAC1 has been shown to interact directly with transcription factors and has been shown to deacetylate histone proteins H3 and H4. The major assay for measuring the expression or amount of HDAC1 protein currently is the Western blot. This method requires electrophoresis and transfer process, which makes the assay inconvenient, time consuming, and has low throughput. The HDAC1 Assay Kit addresses these problems by using a unique procedure to measure amount of HDAC1. The kit has following features:

- The fastest procedure, which can be finished within 3 hours.
- Innovative colorimetric assay to semi-quantitatively measure HDAC1 amount with no need of electrophoresis.
- Strip microplate format makes the assay flexible: manual or high throughput analysis.
- Simple, reliable, and consistent assay conditions.

B. Test Principle

The HDAC1 Assay Kit is designed for measuring total HDAC1 amount from tissues or cells. In an assay with this kit, the nuclear proteins containing HDAC1 are stably coated on the strip wells. The HDAC1 is recognized with highaffinity specific antibody. The amount of HDAC1 can be quantified through HRP conjugated secondary antibody-color development system and is proportional to the intensity of color development.



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 HDAC1 levels from various fresh tissues and cultured mammalian cells.

Material and Method

A. List of component

	96 assays
HA1 (10X wash buffer)	**22 ml**
HA2 (HDAC assay buffer)	**2 ml**
HA3 (blocking buffer)	**20 ml**
HA4 (capture antibody 200 µg/ml)*	**26 µl**
HA5 (detection antibody 200 µg/ml)*	**20 µl**
HA6 (developing solution)	**12 ml**
HA7 (stop solution)	**6 ml**
HDAC1 control (100 ng/µl)*	**32 µl**
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 HDAC1 control and HA5 at –20℃. Store HA3, HA4, HA6 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 (the strip wells can be broken off). Leave these strip wells 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 HA1 with distilled water (pH 7.2-7.5) to the 1 X HA1.
3. Adjust protein concentration to 0.4-1 µg/µl with HA2 and add 5 µl (2-5 µg) of the protein solution into central area of each well. Spread out the solution over the bottom of the strip well by pipetting the solution up and down several times. Incubate the strip wells at 37℃ (with no humidity) for 60-90 min to evaporate the solution and completely dry the wells. For the blank, add 5 µl of HA2 to the wells. For the positive control, dilute HDAC1 control to 2-30 ng/µl with HA2 and add 5 µl (10-150 ng) of diluted HDAC1 control

solution to the wells.

4. Add 150 µl of HA3 to the dried wells and incubate at 37℃ for 30-45 min.
5. Aspirate and wash each well with 150 µl of 1 X HA1 three times.
6. Dilute the HA4 (at 1:200 ratio) to 1 µg/ml with 1 X HA1. Add 50 µl of diluted HA4 to each assay strip well and 2 control strip wells. Incubate the samples at room temperature for 60 min on a orbital shaker (50-100 rpm).
7. Aspirate and wash each well with 150 µl of 1 X HA1 four times.
8. Dilute the HA5 (at 1:1000 ratio) to 0.2 µg/ml with 1 X HA1. Add 50 µl of diluted HA5 to each strip well and incubate at room temperature for 30 min.
9. Aspirate and wash each well with 150 µl of 1 X HA1 four times. Allow 3 min for last wash.
10. Add 100 µl of HA6 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).
11. Add 50 µl of HA7 to each well and read absorbance on microplate reader at 450 nm.
12. Calculate HDAC1 level.

$$\text{HDAC1 level (OD/ml)} = (\text{sample OD} - \text{blank OD}) \times \text{sample dilution}$$

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:

$$\text{Amount (ng/mg protein)} = \frac{\text{OD (sample} - \text{blank)}}{\text{slope}} \times 1000$$

Troubleshooting

- **No Signal for Both the Positive Control and the Samples**

Reagents are added incorrectly.	Check if reagents are added in order and if some steps of the procedure are omitted by mistake.
Reagents are added incorrectly.	Ensure the well is incubated with no humidity and dry before adding block buffer.
The well is incorrectly washed before protein coating.	Ensure the well is not washed before adding positive control or protein extracts.
Incubation time and temperature is incorrect.	Ensure the incubation time and temperature described in the protocol are correctly followed.

- **No Signal or Very Weak Signal For Only the Positive Control**

The HDAC1 control protein is insufficiently added to the well.	Ensure sufficient amount of control protein is added.
The positive control is degraded due to incorrect storage.	Follow the guidance in the protocol for storage of positive control.

- **No Signal for Only the Sample**

The protein amount is added into well insufficiently.	Ensure extract contains enough amount of proteins.
Nuclear extracts are incorrectly stored.	Ensure the nuclear extracts are stored at –80°C.

- **High Background Present for the Blank**

The well is not washed enough.	Check if wash at each step is performed according to the protocol.
Contaminated by the positive control.	Ensure the well is not contaminated by adding the control protein or from using control protein contaminated tips.
Overdevelopment.	Decrease development time in Step 10.