HDAC8 Assay Kit

Catalog Number KA0631

96 assays

Version: 01

Intended for research use only

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Introduction

Intended Use

For measuring HDAC8 levels from various fresh tissues and cultured mammalian cells.

Features:

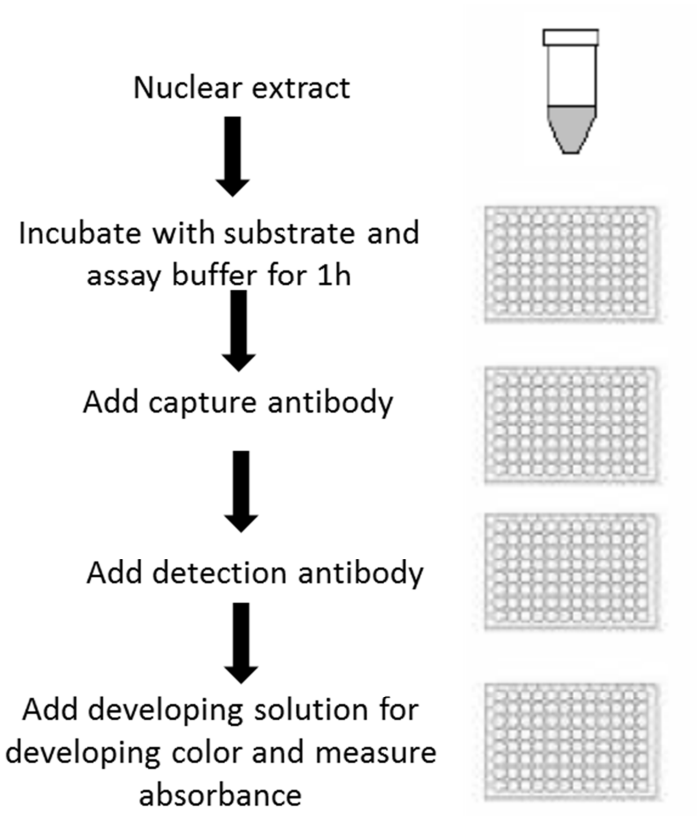
- ✓ The fastest procedure, which can be finished within 3 hours.
- ✓ Innovative colorimetric assay to semi-quantitatively measure HDAC8 amount with no need of electrophoresis.
- ✓ Strip microplate format makes the assay flexible: manual or high throughput analysis.
- ✓ Simple, reliable, and consistent assay conditions

Background

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. HDAC8 is a class I histone deacetylase containing 377 amino acid residues. HDAC8 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 HDAC8 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 HDAC8 Assay Kit addresses these problems by using a unique procedure to measure amount of HDAC8.

Principle of the Assay

The HDAC8 Assay Kit is designed for measuring total HDAC8 amount from tissues or cells. In an assay with this kit, the nuclear proteins containing HDAC8 are stably coated on the strip wells. The HDAC8 is recognized with highaffinity specific antibody. The amount of HDAC8 can be quantified through HRP conjugated secondary antibody-color development system and is proportional to the intensity of color development.


Nuclear extract
Incubate with substrate and assay buffer for 1h
Add capture antibody
Add detection antibody
Add developing solution for developing color and measure absorbance

General Information

Materials Supplied

List of component

Component	Amount
HC1 (10X wash buffer)	22 ml
HC2 (HDAC assay buffer)	2 ml
HC3 (blocking buffer)	20 ml
HC4 (capture antibody 200 µg/ml)*	26 µl
HC5 (detection antibody 200 µg/ml)*	20 µl
HC6 (developing solution)	12 ml
HC7 (stop solution)	6 ml
HDAC8 control (100 ng/µl)*	24 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.

Storage Instruction

Upon receipt, store HC5 and HDAC8 control at –20℃.

Store HC3, HC4, HC6 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.

Note:Check if wash buffer, HC1, contains salt precipitates before using. Ifso, warm (at room temperature or 37℃) and shake the buffer until the salts are re-dissolved.

Materials Required but Not Supplied

- ✓ Orbital shaker
- ✓ Pipettes and pipette tips
- ✓ Microplate reader
- ✓ 1.5 ml microcentrifuge tubes

Precautions for Use

To avoid cross-contamination, carefully pipette the sample or solution into the strip wells. Use aerosolbarrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

Assay Protocol

Assay Procedure

1. Prepare nuclear extracts by using you own successful method. Nuclear extracts can be used immediately or stored at -80 ºC 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 4ºC). Dilute 10 X HC1 with distilled water (pH 7.2-7.5) to the 1 X HC1.
3. Adjust protein concentration to 0.4-1 µg/µl with HC2 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 HC2 to the wells. For the positive control, dilute HDAC8 control to 2-30 ng/µl with HC2 and add 5 µl (10-150 ng) of diluted HDAC8 control solution to the wells.
4. Add 150 µl of HC3 to the dried wells and incubate at 37℃ for 30-45 min.
5. Aspirate and wash each well with 150 µl of 1 X HC1 three times.
6. Dilute the HC4 (at the 1:200 ratios) to 1 µg/ml with 1 X HC1. Add 50 µl of diluted HC4 to each assay strip well and 2 control strip wells. Incubate the samples at room temperature for 60 min on an orbital shaker (50-100 rpm).
7. Aspirate and wash each well with 150 µl of 1 X HC1 four times.
8. Dilute the HC5 (at the 1:1000 ratios) to 0.2 µg/ml with 1 X HC1. Add 50 µl of diluted HC5 to each strip well and incubate at room temperature for 30 min
9. Aspirate and wash each well with 150 µl of 1 X HC1 four times. Allow 3 min for last wash.
10. Add 100 µl of HC6 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 HC7 to each well and read absorbance on microplate reader at 450 nm

Data Analysis

Calculation of Results

Calculate HDAC8 level:

$$\text{HDAC8 level}\left(\frac{\text{OD}}{\text{ml}}\right) = (Sample\ OD - Blank\ OD) \times sample\ dilution$$

For an accurate calculation, plot OD value versus amount of HDAC8 control and determine the slope as delta OD/ng.

Calculate HDAC activity using the following formula:

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

Resources

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.
The well is not completely dried.	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 positive control 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℃.

- 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.