HDAC Activity/Inhibition Assay Kit (Fluorometric)

Catalog Number KA0627

96 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

The HDAC Activity/Inhibition Assay Kit (Fluorometirc) is very suitable for measuring HDAC activity/inhibition from a broad range of species including mammalian cells/tissues, plants, and bacteria.

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

The HDAC Activity/Inhibition Assay Kit (Fluorometirc) uses a proprietary and unique procedure to measure HDAC activity/inhibition with the following features:

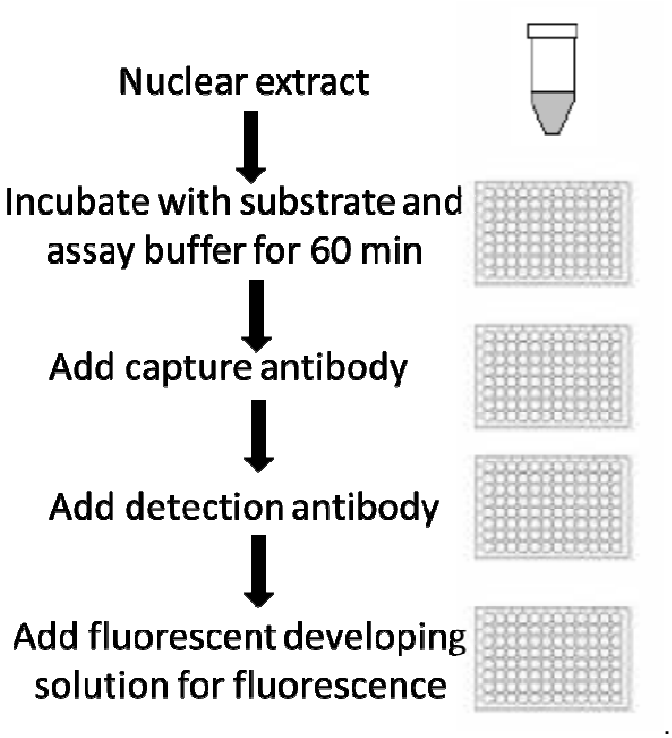
- ✓ Fast procedure, which can be finished within 3 hours.
- ✓ Innovative fluorescent assay without need for radioactivity, extraction, and chromatography.
- ✓ Direct measurement of HDAC activity and inhibition with no use of lysyl endopeptidase, thereby avoiding the false inhibitory effect on HDACs and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

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. 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.

Principle of the Assay

The HDAC Activity/Inhibition Assay Kit (Fluorometric) 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 fluorometrically quantified.


Nuclear extract
Incubate with substrate and assay buffer for 60 min
Add capture antibody
Add detection antibody
Add fluorescent developing solution for fluorescence

General Information

Materials Supplied

List of component

Component	Amount
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 Assay standard, 20 µg/ml)*	50 µl
H6 (capture antibody 1000 µg/ml)*	10 µl
H7 (detection antibody 200 µg/ml)*	20 µl
Fluoro-developer	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.

Storage Instruction

Upon receipt, store H3, H4, H5 and H7 at –20℃ away from light. Store H1, H6, fluoro-developer and 8 well assay strips at 4℃ away from light. Store H2 at room temperature. The components of the kit should be stable for 6 months when stored properly.

Note: Check if wash buffer H1 contain salt precipitates before using. If so, warm (at room temperature or 37℃) and shake the buffers until the salts are redissolved.

Materials Required but Not Supplied

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

Assay Protocol

Assay Procedure

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 a 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 5-6 times.
11. Add 50 µl of fluoro-developer into the wells and incubate at room temperature for 1-5 min away from light. The color in the standard wells containing the higher concentrations may turns slightly pink during this period. Measure and read fluorescence on fluorescence microplate reader at $530_{EX}/590_{EM}$ nm.
    Note: If the strip well frame does not fit the fluorescence reader, transfer the solution to the standard 96-well microplate and read fluorescence at $530_{EX}/590_{EM}$ nm.

Data Analysis

Calculation of Results

Calculate HDAC activity or inhibition. For simple calculation:

$$\text{HDAC activity (RFU/h/µg)} = \frac{[\text{RFU (control – blank)} - \text{RFU (sample – blank)}]}{\text{Reaction time (h)} \times \text{protein amount added}}$$

Inhibition % =

$$1 - \frac{[\text{RFU (control – blank)} - \text{RFU (inhibitor sample – blank)}]}{[\text{RFU (control – blank)} - \text{RFU (no inhibitor sample – blank)}]} \times 100\%$$

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:

$$\text{Activity (ng/h/µg)} = \frac{[\text{RFU (control-blank)} - \text{RFU (sample – blank)}]}{\text{slope} \times \text{h} \times \text{protein amount added}}$$

Resources

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.

Plate Layout

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								