HAT Activity/Inhibition Assay Kit (Colorimetric)

Catalog Number KA0626

96 assays

Version: 03

Intended for research use only

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Introduction

Intended Use

The HAT Activity/Inhibition Assay Kit (Colorimetric) is very suitable for measuring HAT activity/inhibition from a broad range of species including mammalian cells/tissues, plants, and bacteria.
The HAT Activity/Inhibition Assay Kit (Colorimetric) uses a proprietary and unique procedure to measure HAT activity/inhibition with the following features:

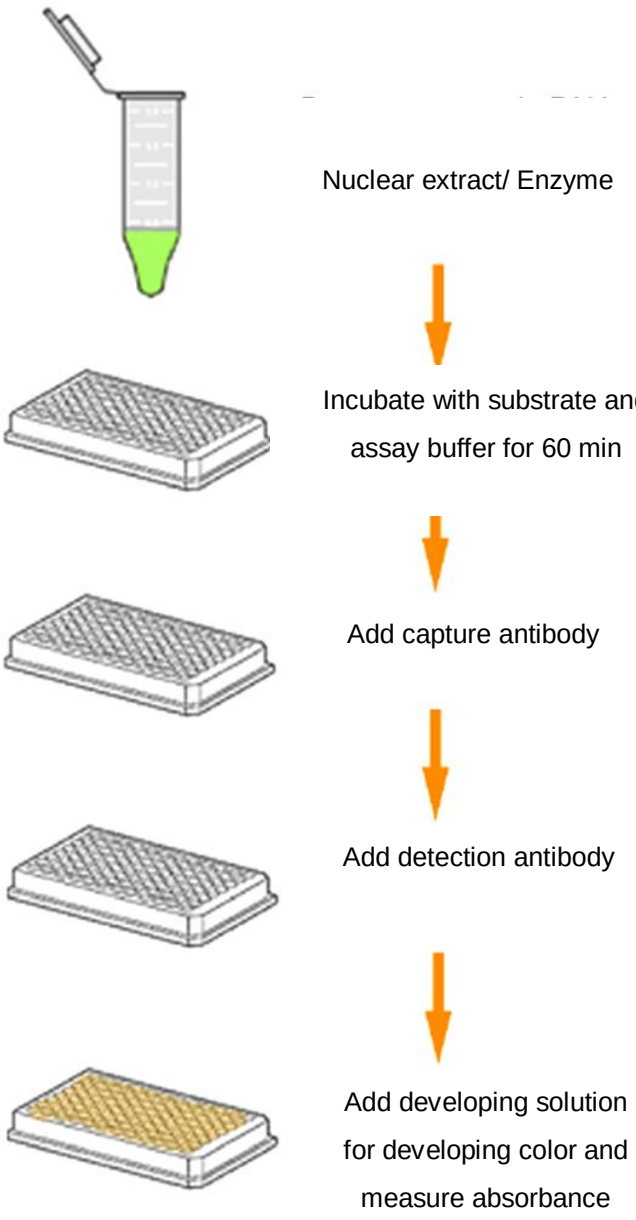
- ✓ Fast procedure, which can be finished within 3 hours.
- ✓ Innovative colorimetric assay without the use of radioactivity, extraction, or chromatography.
- ✓ Direct measurement of HAT activity and inhibition by quantifying the amount of acetylated histone substrate, thereby avoiding the false inhibitory effect on HATs.
- ✓ Strip microplate format makes the assay flexible: manual or high throughput analysis.
- ✓ Simple, reliable, and consistent assay conditions.

Background

Histone acetylases (HATs) play a critical role in transcriptional activation of gene expression in eukaryotic cells through modifying N-terminal lysine residues of histones by the addition of an acetyl group from acetyl coenzyme A. More than 20 HATs have been identified and these HATs can be classified into five families: GNAT1, MYST, TAFII250, P300/CBP, and nuclear receptor co-activators such as ACTR. HAT activation or inhibition displays significant effects on several diseases ranging from neurodegenerative disorders to cancer. The impact of HATs on cellular physiology and disease would benefit from the identification of specific pharmacological inhibitors. Several HAT inhibitors are developed to date. There are several methods used for measuring HAT activity/inhibition. However, most of these methods available so far are to indirectly measure HAT activity through detecting generation of free CoA or CoA-SH, which may cause the measured HAT activity and inhibitory effects of inhibitors to be less accurate.

Principle of the Assay

The HAT Activity/Inhibition Assay Kit (Colorimetric) is designed for measuring total HAT activity/inhibition. In an assay with this kit, the unique histone substrate is stably captured on the strip wells. Active HATs bind to and acetylate histone substrate. The acetylated substrate can be recognized with a high affinity anti-acetylated histone antibody. The ratio or amount of the acetylated histone, which is directly proportional to HAT enzyme activity, can then be colorimetrically quantified through an ELISA-like reaction.



Schematic Procedure for using the HAT Activity/Inhibition Assay Kit (Colorimetric)

General Information

Materials Supplied

List of component

Component	Amount
HT1 (10X Wash Buffer)	28 ml
HT2 (HAT Substrate, 20 µg/ml)*	100 µl
HT3 (HAT Assay Standard, 20 µg/ml)*	50 µl
HT4 (Acetyl Co-A, 30 mM)*	20 µl
HT5 (HAT Assay Buffer)	3 ml
HT6 (Capture Antibody, 100 µg/ml)*	50 µl
HT7 (Detection Antibody, 200 µg/ml)*	20 µl
HT8 (Developing Solution)	12 ml
HT9 (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.*

Storage Instruction

Upon receipt: (1) Store HT2, HT3, HT4, and HT7 at –20°C away from light; (2) Store HT1, HT6, HT8, and 8-Well Assay Strips at 4°C away from light; (3) Store all remaining components at room temperature. All components of the kit are stable for 6 months from date of shipment, when stored properly.

Note: Check if wash buffer, HT1, contains salt precipitates before using. If so, warm (at room temperature or 37°C) 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

The HAT Activity/Inhibition Assay Kit (Colorimetric) is for research use only and is not intended for diagnostic or therapeutic application.

Assay Protocol

Assay Procedure

1. Prepare nuclear extracts by using your own successful method. Nuclear extracts can be used immediately or stored at -80°C 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°C). Dilute HT1 with distilled water (pH 7.2-7.5) at a 1: 10 ratio (ex: 1 ml of HT1 + 9 ml of distilled water).
3. Dilute HT2 at the 1:50 ratios with diluted HT1, and then add 50 µl of the diluted HT2 into each well, except the wells for standard curve. For preparation of standard curve, add 50 µl of diluted HT1 into the wells (no HT2), followed by add 1 µl of HT3 at different amount (0.1 – 10 ng); HT3 can be diluted with diluted HT1 to achieve your different concentration points. 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 diluted HT1 three times.
5. Dilute HT4 at the 1:20 ratios with HT5. Add 26 µl of HT5, 2 µl of the diluted HT4 and 2 µl of nuclear extracts (4-20 µg) or HAT enzymes to each strip well. Mix, cover the strip wells, and incubate at 37°C for 30-60 min. For the standard curve, add 2 µl of HT5 instead of nuclear extracts or HAT enzymes. For HAT inhibition, add 2 µl of different amounts of the tested inhibitors and reduce HT5 volume to 24 µl. For the blank, add 30 µl of HT5 into the blank wells.
6. Aspirate and wash each well with 150 µl of diluted HT1 three times.
7. Dilute HT6 (at the 1:100 ratios) to 1 µg/ml with diluted HT1. Add 50 µl of the diluted HT6 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 diluted HT1 four times.
9. Dilute HT7 (at the 1:1000 ratios) to 0.2 µg/ml with diluted HT1. Add 50 µl of the diluted HT7 to each strip well and incubate at room temperature for 25-30 min.
10. Aspirate and wash each well with 150 µl of diluted HT1 four to five times.
11. Add 100 µl of HT8 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 HT9 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.

Data Analysis

Calculation of Results

✓ Calculate HAT activity or inhibition.

- For simple calculation:

$$\text{HAT activity (OD/h/mg protein)} = \frac{\text{OD (untreat sample - blank)}}{\text{hour x protein amount } (\mu\text{g}) \text{ added into the assay}} \text{ x } 1000$$

$$\text{Inhibition \%} = (1 - \frac{\text{OD (inhibitor sample - blank)}}{\text{OD (no inhibitor control - blank)}}) \text{ x } 100\%$$

For an accurate calculation, plot OD value versus amount of HT3 and determine the slope as delta OD/ng.

- Calculate HAT activity using the following formula:

$$\text{Activity (ng/h/mg)} = \frac{\text{OD (untreated sample - blank)}}{\text{slope x hour x protein amount } (\mu\text{g}) \text{ added into the assay}} \text{ x } 1000$$

Resources

Troubleshooting

✓ No Signal for the Sample

The protein sample is not properly extracted.	Ensure the 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 lost 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 followed correctly.
Absence of HAT activity in the sample due to treatment.	N/A

✓ High Background Present for the Blank

The well is not washed sufficiently.	Check if wash at each step is performed according to the protocol.
Overdevelopment.	Decrease development time in Assay Procedure.

Plate Layout

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