ab65352

Histone Acetyltransferase Activity Assay Kit

Instructions for Use

For the rapid, sensitive, and accurate measurement of Histone Acetyltransferase activity in cell nuclear extracts, purified protein, and tissue lysates.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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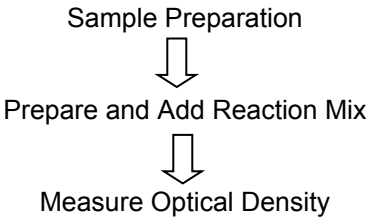
1. Overview

Histone acetyltransferases (HATs) have been implicated to play a crucial role in various cellular functions, such as gene transcription, differentiation, and proliferation. Abcam's Histone Acetyltransferase Activity Assay Kit offers a convenient, nonradioactive system for a rapid and sensitive detection of HAT activity in mammalian samples.

The kit utilizes active Nuclear Extract (NE) as a positive control and acetyl-CoA as a cofactor. Acetylation of peptide substrate by active HAT releases the free form of CoA which then serves as an essential coenzyme for producing NADH. NADH can easily be detected spectrophotometrically upon reacting with a soluble tetrazolium dye. The detection can be continuous and suitable for kinetic studies. The kit provides a simple, straightforward protocol for a complete assay.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
2X HAT Buffer/2X HAT Assay Buffer	7.5 mL
Substrate IV/HAT Substrate I	1 vial
HAT Peptide Substrate Mix/HAT Substrate II	1 vial
NADH Generating Enzyme	800 µL
Nuclear Extract Positive Control/Nuclear Extract (NE, 4 mg/ml)	50 µL
HAT Reconstitution Buffer	1.8 mL

* Store kit at -80°C.

- Nuclear Extract or purified protein samples can be tested using this kit.
- Samples containing DTT, Coenzyme A, and NADH should be avoided, as these compounds strongly interfere with the reactions.

- Using U-shaped 96-well plates may increase signal up to 40% in comparison to flat bottom plates.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent or colorimetric microplate reader
- 96 well plate
- Orbital shaker

4. Assay Protocol

The Histone Acetyltransferase Activity Assay Kit provides an easy and very simple procedure to assay HAT activity (just add reagents to sample preparations, incubate and read).

Unlike the conventional radioisotope method, the assay continuously measures HAT activity and thus is suitable for kinetic studies. In addition, the assay is not interfered by the presence of histone deacetylases and therefore, crude nuclear extract can be used directly in the assay.

1. Sample Preparation:

Nuclear Extract Positive Control/Nuclear extract or purified protein: Prepare test samples (50 µg of Nuclear Extract Positive Control/nuclear extract or purified protein) in 40 µl water (final volume) for each assay in a 96-well plate. For background reading, add 40 µl water instead of sample. For positive control, add 10 µl of the NE (Cell Nuclear Extract Positive Control/Nuclear Extract) and 30 µl water.

Tissue lysate: Homogenize tissue sample in dH_2O with 0.1% triton x-100 using a dounce homogenizer (on ice) and then centrifuge at 10,000 g for 5 min at 4°C and use the supernatant for the assay.

Note: We would recommend using Nuclear Extract Positive Control/nuclear extract or purified protein, as crude tissue lysate will have less activity.

2. Assay Mix Preparation:

a) Reconstitute Substrate IV/HAT Substrate I, HAT Peptide Substrate Mix/substrate II with 550 µl HAT Reconstitution Buffer. The HAT Peptide Substrate Mix/Substrate II will be become brown cloudy and milky color. Pipette up and down several times to dissolve. Mix well before use. The reagents are stable for two months at -80°C after reconstitution.

b) Mix enough reagents for the number of assays performed. For each well, prepare a total 68 µl Assay Mix containing:

2X HAT Buffer/2X HAT Assay Buffer	50 µl
Substrate IV/HAT Substrate I	5 µl
HAT Peptide Substrate Mix/HAT Substrate II	5 µl
NADH Generating Enzyme	8 µl

3. Mix the prepared Assay Mix; add 68 µl of Assay Mix to each well, mix to start the reaction.

4. Incubate plates at 37°C for 1-4 hours depending on the color development. Read sample in a plate reader at 440 nm. For kinetic studies, read OD_{440nm} at different times during incubation. Select two time points T1 and T2 that define the linear range and calculate the slope using the equation: $A = \frac{OD2 - OD1}{T2 - T1}$

5. Calculate the molar concentration in mol/L/min using the Beer-Lambert law: $C = \frac{A}{\varepsilon b}$

Where:

A= the slope previously calculated

b = pathlength in cm

ε_{440nm} = 37000 $M^{-1}cm^{-1}$ under the kit assay conditions.

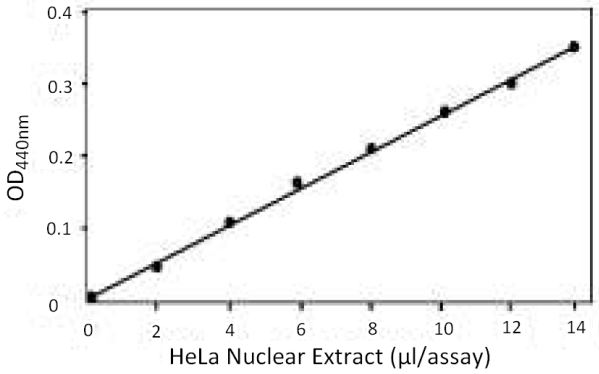
Notes:

a) The yellow color develops slowly, but very steadily and repeatable.

b) Background reading from buffer and reagents (without HAT) is significant, which should be subtracted from the readings of all samples.

c) Multiplying the concentration C calculated at step 5 by the volume in the assay will give an answer in µmol/min, which can be transformed into nmol/min. This can be expressed as relative to mass of sample used by dividing the number by the µg of nuclear extract used per well (nmol/min/µg).

5. Data Analysis



Analyses of HAT Activity in HeLa Nuclear Extract: HeLa nuclear extract in various amounts was incubated with HAT substrate. Activity was analyzed in a micro plate reader at 440 nm according to the kit instructions

6. Troubleshooting

Problem	Reason	Solution
Assay not working	2X HAT Buffer/Assay buffer at wrong temperature	2X HAT Buffer/Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the 2X HAT Buffer/assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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