Colorimetric Histone Acetyltransferase Activity Assay (HAT)

Catslog #8668

100 Tests in 96-well plate

Introduction

Histone acetyltransferases (HATs) catalyze the acetylation of histone proteins by transferring an acetyl group from cofactor acetyl-CoA, and consequently regulate gene expression. The regulation is critical for cellular processes such as cell differentiation and proliferation. Our Colorimetric Histone Acetyltransferase (HAT) Activity Assay Kit offers rapid and sensitive detection of HAT activity or HAT inhibition in mammalian cells/tissue samples. Briefly, acetylation of a peptide substrate by HATs releases the free form of CoA, which can serve as a coenzyme of a NADH generating enzyme to produce NADH. The stoichiometric NADH production can be quantified by spectrophotometric analysis at 440 nm upon reacting with tetrazolium-1 (WST-1), a soluble tetrazolium dye. The HAT assay does not require radioactivity or chromatography and can be applied for kinetic studies. HATs' counteracting enzymes, histone deacetylases (HDACs), do not interfere with the assay, consequently crude nuclear extract or cell lysate samples can be used.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8668a	1	HAT assay buffer	15 mL	-20°C
8668b	1	HAT positive control (cell lysate)	50 µL	-80°C
8668c	1	Developer (10X)	1 mL	-20°C
8668d	1	NADH generating enzyme	0.1 mL	-80°C
8668e	1	WST-1	4 mg	-20°C
8668f	1	Substrates I	1 mL	-20°C
8668g	1	Substrates II	1 mL	-80°C
8668h	1	Cofactors	1 mL	-20°C

Additional Materials Required (Materials Not Included in Kit)

Cell lysis buffer (e.g., 1X RIPA buffer)

96-well flat bottom plate

Product Use

HAT is for research use only. It is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

Shipping and Storage

The product is shipped on dry ice. Upon receipt, components #8668a, 8668c, 8668e, 8668f and 8668h should be stored at -20°C, #8668b, 8668d and 8668g should be stored at -80°C. Protect

from light. Repeated freeze/thaw cycles should be avoided. Aliquot if necessary. If stored properly, the kit is good for up to 6 months.

Quality Control

Serially diluted HAT positive control samples are measured continuously using this kit for a total of 25 minutes. The detection range of HAT activity is 0.1-2 mUnits/mL.

Procedure (for a 96-well plate)

A. Reagents Preparation

1. Developer solution (1X): dilute developer (10X) (Cat #8668c) in HAT assay buffer (Cat #8668a) (1:10). For example, add 90 µL HAT assay buffer to 10 µL developer (10X) to make 100 µL developer (1X).
2. WST-1 solution: reconstitute each vial of WST-1 (Cat #8668e) with 1 mL HAT assay buffer (Cat #8668a). Vortex briefly and keep in the dark at -20°C until use. For longer storage, aliquot and store the reconstituted WST-1 solution at -80°C, and avoid repeated freeze/thaw cycles.

B. Preparation of test samples, positive control and blank

3. Lyse cell pellet or tissue samples using cell lysis buffer or homogenization of your choice (materials not provided) according to supplier's protocol.
   Important: Compounds DTT, CoA and NADH will interfere with the reactions, and should be avoided when preparing the samples.
4. Add 10 µL test sample/well of a 96-well flat bottom plate. Test samples should be prepared to ensure the final HAT activity in well is within the kit detection range of 0.1-2 mUnits/mL. Serial dilution of test samples is recommended for this purpose.
   Example of 7-well 1:2 serial dilution: in first well, add 20 µL test sample. In wells #2-7, add 10 µL HAT assay buffer (Cat #8668a) to each. Transfer 10 µL sample from well #1 to well #2, mix well by pipetting up-and-down several times and then transfer 10 µL from well #2 to well #3, and repeat until all 7 wells are processed. Remove 10 µL sample from well #7 to make the final volume 10 µL in each well.

Well #	1	2	3	4	5	6	7
Dilution factor	1	2	4	8	16	32	64

5. Prepare a blank by adding 10 µL HAT assay buffer (Cat #8668a) into one well of the 96-well flat bottom plate, and a positive control by adding 1 µL HAT positive control (Cat #8668b) and 9 µL HAT assay buffer (Cat #8668a) into another well of the 96-well flat bottom plate.

C. Working reagent preparation and measurements

6. Prepare appropriate volume of HAT assay working reagent based on the number of samples to be measured. For each well of reaction, prepare working reagent by mixing 39 µL HAT assay buffer (Cat #8668a), 10 µL developer solution (1X), 1 µL NADH generating enzyme (Cat #8668d), 10 µL WST-1 solution, 10 µL substrates I (Cat #8668f), 10 µL substrates II (Cat #8668g) and 10 µL cofactors (Cat #8668h).
7. Add 90 µL of working reagent mix into each well of the 96-well plate containing the test samples, HAT positive control and blank. Mix well and record OD_{440nm} every 5 minutes for a total of 25 minutes.

D. Calculations

8. Background reading from blank (without HAT) is significant and should be subtracted from the readings of all samples to get ΔOD_{440nm} of each sample.

$$\Delta OD_{440nm,\ sample} = OD_{440nm,\ sample} - OD_{440nm,\ blank}$$

9. Determine the change in absorbance ΔOD_{440nm}/min by plotting the ΔOD_{440nm} value at as a function of reaction time to obtain the slope of the linear portion of the curves. Figure 1 shows an example of assaying HAT activity using this kit.

10. Calculate HAT activity in each well using the following formula specifically adjusted for this kit:

$$\text{HAT activity (U/ml)} = \frac{\Delta OD_{440nm}/\text{min} \times 100\ \mu l}{11.53\ mM^{-1} \times 10\ \mu l} \times \text{sample dilution}$$

HAT activity unit definition: One unit of HAT activity is the amount of enzyme that will generate 1.0 µmole of CoA per minute at 25 °C under kit assay conditions. One unit of HAT activity makes 1.0 µmol of WST-1 to WST-1:formazan per minute at pH 7.4 at 25 ºC.

The extinction coefficient of WST-1:formazan at 440 nm: 37 $mM^{-1}cm^{-1}$.

E. Example calculations

HAT activity of seven cell lysate samples is measured following the procedure of this kit. All samples were diluted 1:8 first, then 10 µL of each diluted sample was measured in a 100 µL final volume. The slope of ΔOD_{440nm}/min of each sample is shown in Figure 1 and Table 1. The calculated HAT activity is shown in Table 1.

Table 1. Calculated HAT activity of seven test samples.

Sample #	1	2	3	4	5	6	7
Slope of ΔOD_{440nm}/min	0.0211	0.0179	0.0138	0.0092	0.0072	0.0052	0.0025
Total HAT activity in well (µUnits)	190	161	124	82.9	64.9	46.8	22.5
Sample HAT activity (mUnits/mL)	152	129	99.2	66.3	51.9	37.4	18.0

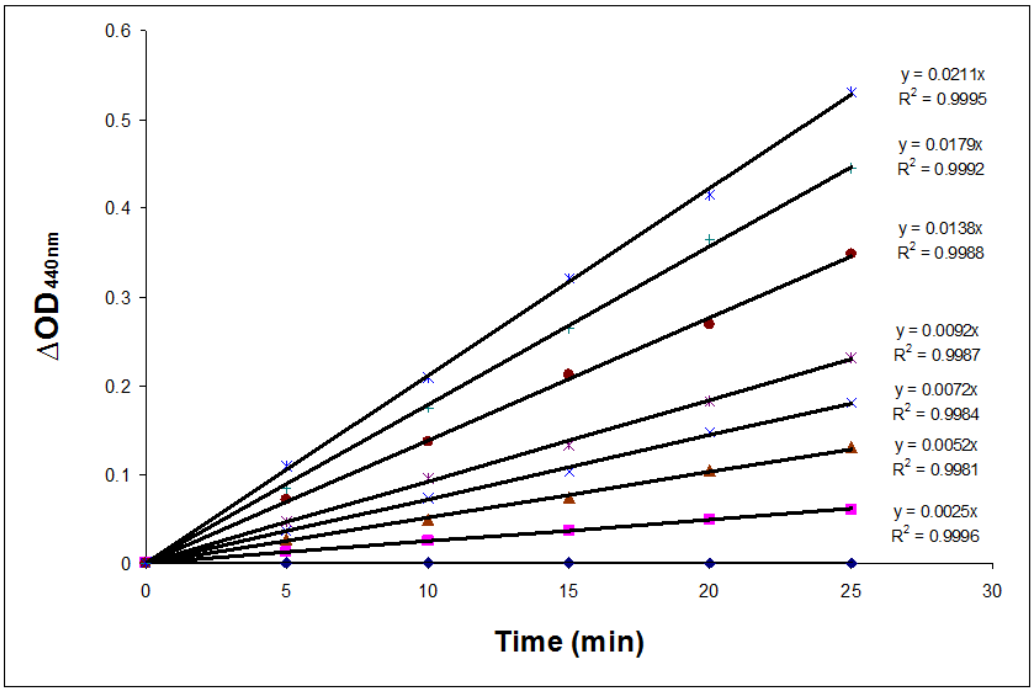

Figure 1. Absorbance change at 440 nm over 25 minutes of seven test samples using this kit.

Example calculation of HAT activity of test sample #1:

Slope (ΔOD_{440nm}/min) = 0.0211

HAT activity (U/mL) = [(0.0211 x 100 µl) / (11.53 mM^{-1} x 10 µl)] x 8 = 0.15 U/ml