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ab239713 Histone Acetyltransferase (HAT) Activity Assay Kit (Fluorometric, Histone H4)

For the detection of HAT activity in cells and tissue.

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

Table of Contents

1. Overview

Histone Acetyltransferase (HAT) Activity Assay Kit (Fluorometric, Histone H4) (ab239713) utilizes Acetyl CoA and H4 histone peptide as substrates. In this assay, HAT enzyme catalyzes the transfer of acetyl groups from Acetyl CoA to the histone peptide, thereby generating two products - acetylated peptide and CoA-SH. The CoA-SH reacts with the developer and Probe to generate a product that is detected fluorometrically at Ex/Em = 535/587 nm.

The assay can detect HAT activity equivalent to as low as 1.5 U of p300 in a variety of samples.

2. Protocol Summary

Prepare tissue or cell samples, background controls, positive controls, and add to appropriate wells.

Prepare standard curve.

Prepare reaction mix and add to sample, background, standards and positive control wells.

Read fluorescence (Ex/Em = 535/587 nm) in kinetic mode at 30°C for 30-60 min.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -80°C in the dark immediately upon receipt check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
Acetyl CoA (Lyophilized)	1 vial	-80°C
CoA Standard (Lyophilized)	1 vial	-80°C
Developer	100 µL	-20°C
H4 Peptide (Lyophilized)	1 vial	-80°C
HAT Assay Buffer	25 mL	-80°C
Positive Control (HeLa Nuclear Extract)	40 µL	-80°C
Probe	200 µL	-20°C
Substrate Mix (Lyophilized)	1 vial	-80°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 96-well plate with flat bottom
- Multi-well spectrophotometer capable of fluorescence detection

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 HAT Assay Buffer:

Warm to room temperature before use.

6.2 Acetyl CoA:

Reconstitute with 220 µL deionized water. Make 20 µL aliquots and store at -80°C. Stable at -80°C for two months. Avoid repeated freeze/thaw. Keep on ice while in use.

6.3 H4 Peptide:

Reconstitute with 420 µL HAT Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -80°C. Avoid repeated freeze/thaw. Use within two months. Keep on ice while in use.

6.4 Substrate Mix:

Reconstitute with 1.1 ml HAT Assay Buffer. Pipette up and down to dissolve. Store at -80°C. Use within two months.

6.5 Developer:

Store at -20°C. The solution is very viscous and difficult to pipette accurately. Immediately prior to use, take the required volume of developer and dilute 1:1 with an equal volume of HAT Assay Buffer.

6.6 Probe:

Warm to room temperature and mix well before use. Store at -20°C.

6.7 CoA Standard:

Reconstitute with 100 µL HAT Assay Buffer to generate 100 mM solution and mix completely. Aliquot and store at -80°C. Avoid repeated freeze/thaw. Use within two months.

6.8 Positive Control:

Aliquot and store at -80°C. Avoid repeated freeze/thaw. Use within two months.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Diluted CoA Standard is unstable. Discard the diluted Standard after use.

7.1 Dilute CoA Standard to 1 mM by adding 10 µL of 100 mM CoA Standard to 990 µL of HAT Assay Buffer.

7.2 Dilute further to 0.1 mM by adding 10 µL of 1 mM CoA Standard to 90 µL of HAT Assay Buffer.

7.3 Add 0, 2, 4, 6, 8 and 10 µL of 0.1 mM CoA Standard into a series of wells in a 96 well plate to generate 0, 200, 400, 600, 800 and 1000 pmol/well of CoA Standard.

7.4 Adjust the volume to 50 µL/well with HAT Assay Buffer.

Standard #	1 mM CoA Standard (µL)	HAT Assay Buffer (µL)	CoA Standard pmol/well
1	0	50	0
2	2	48	200
3	4	46	400
4	6	44	600
5	8	42	800
6	10	40	1000

8. Sample Preparation

8.1 Prepare nuclear extract using whichever method you prefer.

8.2 Add 2-10 µL of sample and make up the volume to 50 µL with HAT Assay Buffer.

8.3 Add 50 µL HAT Assay Buffer to one of the wells as Background Control.

8.4 For immunoprecipitated (IP) samples, use the beads containing the HAT-Antibody complex (unknown sample) or beads with non-specific immunoglobulin from the same host species as the HAT Antibody (Background Control), directly for the assay.

8.5 Make up the volume to 50 µL with HAT Assay Buffer.

8.6 For Positive Control, add 2-4 µL of HeLa Nuclear Extract into desired well(s) and make up the volume to 50 µL with HAT Assay Buffer.

Δ Note: For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

Δ Note: Reducing agents such as Dithiothreitol (DTT) and β mercaptoethanol will interfere with the assay. Make sure samples are free of reducing agents prior to use in the assay.

Δ Note: We recommend using protease inhibitor cocktail and protein A/G Sepharose for the immunoprecipitation.

Δ Note: Endogenous histones present in nuclear extracts will contribute to total HAT activity.

9. Assay Procedure

9.1 Mix enough reagents for the number of assays to be performed. Add reagents in the order shown. For each well, prepare 50 µL Mix containing:

Component	Reaction Mix µL
HAT Assay Buffer	30
H4 Peptide	4
Substrate Mix	10
Diluted Developer	2
Probe	2
Acetyl CoA	2

9.2 Add 50 µl of the reaction mix to each well containing the Samples, Background Control, Standards and Positive Control. Mix well.

9.3 Read fluorescence (Ex/Em = 535/587 nm) in kinetic mode at 30°C for 30-60 min. Choose two time points (T1 and T2) in the linear range of the plot and obtain the corresponding RFU for Sample (RS1 and RS2) and sample background (RB1 and RB2).

10. Data Analysis

10.1 Subtract 0 Standard reading from all Standard readings.
Δ Note: The CoA Standards will show some drift.

10.2 Extrapolate the linear portion of the time curve for each Standard to the Y axis to obtain the Y intercept.

10.3 Plot the Standard Curve using the corrected intercept values.

10.4 Calculate the HAT Activity of the test sample ΔRFU = (RS2 – RS1) – (RB2 – RB1).

10.5 Apply the ΔRFU to the Standard Curve to get B pmol of CoA formed during the reaction time (ΔT = T2 T1).

Sample HAT Activity =

$$\frac{B}{\Delta T \, x \, V} x \, D$$

= pmol/min/ml = µU/ml.

Where:

B is CoA amount from Standard Curve (pmol).
T is reaction time (min).
V is the Sample volume added into the reaction well (ml).
D is the dilution factor.

Sample HAT Activity can also be expressed in µU/µg of protein.

Unit Definition: One unit of HAT activity is the amount of enzyme that generates 1.0 µmol of CoA per min. at 30°C under kit assay conditions.

11. Typical Data

Typical data provided **for demonstration purposes only**.

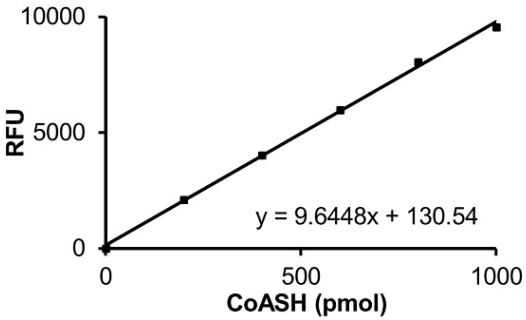

Figure 1. CoASH standard curve.

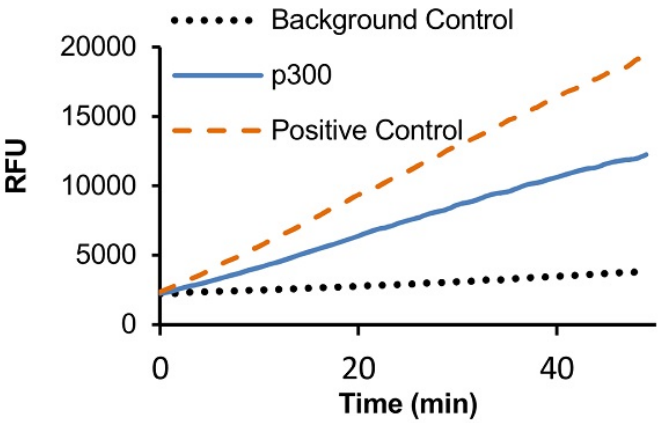

Figure 2. Measurement of HAT Activity: p300 (72 ng) or HeLa nuclear extract (positive control) as per kit protocol.

12. Notes

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