3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

HAT Activity Fluorometric Assay Kit

Catalog Number **EPI002**
Storage Temperature –70 °C

TECHNICAL BULLETIN

Product Description

Histone acetyltransferases (HATs) are enzymes that acetylate histone substrates resulting in important regulatory effects on chromatin structure and assembly, and gene transcription. Modifications of these proteins by HATs play an important role in the control of gene expression and their dysregulation has been linked to cancer, neurodegeneration, and other diseases.

The HAT Activity Fluorometric Assay Kit utilizes acetyl coenzyme A (CoA) and H3 histone peptide as substrates. In this assay, the 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 to generate a product that is detected fluorometrically (λ_{ex} = 535/λ_{em} = 587 nm).

The assay can detect HAT activity as low as 6 milliunits in a variety of samples including nuclear extracts and recombinant enzymes. One unit of HAT activity is the amount of enzyme that will generate 1.0 μmole of CoA per minute at 25 °C using kit assay conditions.

Components

The kit is sufficient for 100 assays in 96 well plates.

HAT Assay Buffer (Wide Mouth Bottle) Catalog Number EPI002A	25 mL
Acetyl CoA (Red Cap) Catalog Number EPI002B	1 vl
H3 Peptide (Brown Cap) Catalog Number EPI002C	1 vl
Substrate Mix (Green Cap) Catalog Number EPI002D	1 vl
Developer (Orange Cap) Catalog Number EPI002E	100 μL
Fluorescent Probe (Blue Cap) Catalog Number EPI002F	200 μL
CoA Standard (Yellow Cap) Catalog Number EPI002G	1 vl
Positive Control (Violet Cap) Catalog Number EPI002H	40 μL

Reagents and equipment required but not provided.

- 96 well flat-bottom plate – white plates with clear bottoms are preferred for this assay
- Fluorescence multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on dry ice and storage at –70 °C, protected from light, is recommended. Store Developer and Fluorescent Probe at –20 °C after thawing.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles. Read the entire protocol before performing the assay.

Warm HAT Assay Buffer to room temperature before use.

Acetyl CoA – Reconstitute with 220 μL of water. Store as 20 μL aliquots at –70 °C. Stable at –70 °C for two months. Keep on ice while in use.

H3 Peptide – Reconstitute with 420 μL of HAT Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at –70 °C. Stable at –70 °C for two months. Keep on ice while in use.

Substrate Mix – Reconstitute with 1.1 mL of HAT Assay Buffer. Pipette up and down to dissolve completely. The reagent is stable at –70 °C for two months after reconstitution.

Developer – 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. Store at –20 °C.

Fluorescent Probe – Warm to room temperature and mix well before use. Store at –20 °C.

CoA Standard – Reconstitute with 100 µL of HAT Assay Buffer to generate 100 mM solution and mix completely. Aliquot and store at –70 °C. Stable at –70 °C for two months.

Positive Control (HeLa Nuclear Extract): Aliquot and store at –70 °C. Stable at –70 °C for two months.

Procedure

Sample Preparation

1. Prepare samples by diluting 2–10 µL of nuclear extract with HAT Assay Buffer to a final volume of 50 µL per well in a 96 well plate.
2. For background reading, prepare a well with 50 µL of HAT Assay Buffer.
3. For a positive control, dilute 2–4 µL of Positive Control (HeLa Nuclear Extract) in HAT Assay Buffer to a final volume of 50 µL.

Notes: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Dithiothreitol (DTT) and β-mercaptoethanol will interfere with the assay. Make sure samples are free of DTT and β-mercaptoethanol.

Standard Curve Preparation

Dilute CoA Standard to 1 mM by adding 10 µL of 100 mM CoA Standard to 990 µL of HAT Assay Buffer. Dilute further to 0.1 mM by adding 10 µL of 1 mM CoA Standard to 90 µL of HAT Assay Buffer. 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 1,000 pmole/well of CoA Standard. Adjust the volume to 50 µL/well with HAT Assay Buffer.
Note: Diluted CoA Standard is unstable. Discard the diluted Standard after use.

Assay Reaction

1. Prepare sufficient Reaction Mix for the number of assays to be performed. Add reagents in the order shown in Table 1. For each assay, prepare 50 µL of Reaction Mix containing:

Table 1.
Reaction Mix

Reagent	**Volume**
HAT Assay Buffer	30 µL
H3 Peptide	4 µL
Substrate Mix	10 µL
Developer	2 µL
Fluorescent Probe	2 µL
Acetyl CoA	2 µL
Total Volume	50 µL

2. Add 50 µL of the Reaction Mix to each well containing the Samples, Background Control, Standards, and Positive Control. Mix well.

3. Read fluorescence (λ_{ex} = 535/λ_{em} = 587 nm) in kinetic mode at 25 °C for 30–60 minutes.

Results

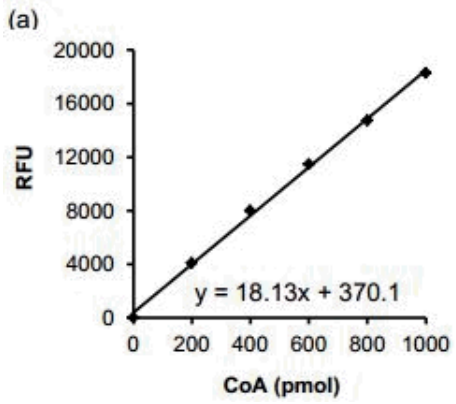
Calculations

Subtract the 0 Standard reading from all Standard readings. Extrapolate the curve for each Standard to the y-axis to obtain the y-intercept. Plot the Standard Curve using the corrected intercept values.

Note: The CoA Standards will show some drift.

Figure 1.

CoA Standard Curve



Choose two time points (T_1 and T_2) in the linear range of the Standard Curve and obtain the corresponding RFU for Sample (R_{S1} and R_{S2}) and Sample Background (R_{B1} and R_{B2}).

$$\Delta RFU = (R_{S2} - R_{S1}) - (R_{B2} - R_{B1}).$$

Using the ΔRFU, determine B (pmole of CoA formed) in the sample during the reaction time ($\Delta T = T_2 - T_1$) from the standard curve.

HAT activity (pmole/min/mL = μunits/mL)

$$\text{HAT Activity} = \frac{B}{\Delta T \times V} \times D$$

B = CoA amount from Standard curve (pmole)
ΔT = Reaction time (minutes)
V = Sample volume added into the reaction well (mL)
D = Dilution factor

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

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 using kit assay conditions.

Figure 2.

HAT Activity in HeLa Nuclear Extract

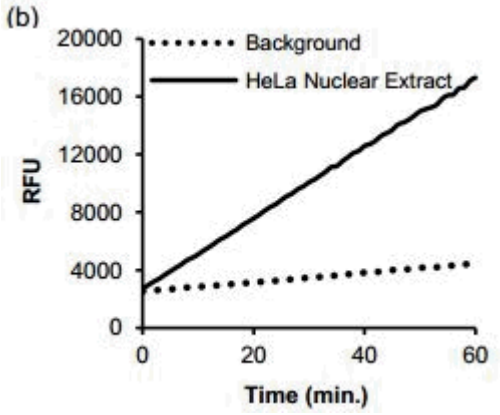
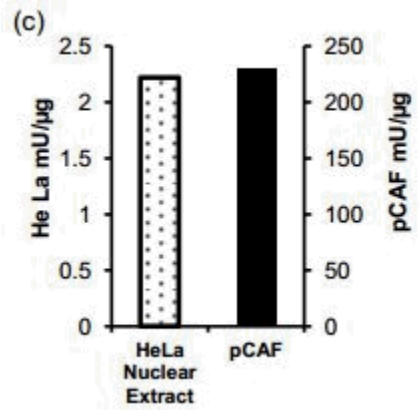


Figure 3.

Specific Activity of HeLa Nuclear Extract and Purified recombinant pCAF



MJK,MAM 11/14–1