# HAT Activity Fluorometric Assay Kit

**(Catalog # BN00600; 100 assays; Store at -80°C)**

## I. Introduction:

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. Assay Genie's HAT Activity Assay Kit utilizes Acetyl CoA and H3 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 to generate a product that is detected fluorometrically at Ex/Em = 535/587 nm. The assay can detect HAT activity as low as 6 mU in a variety of samples.

## II. Applications:

- Measurement of HAT activity in Nuclear Extracts
- Measurement of HAT activity of purified enzyme preparations

## III. Sample Type:

- Nuclear extracts from cells and tissue
- Recombinant enzyme

## IV. Kit Contents:

| Components | BN00600 | Cap Code | Part Number |
|---|---|---|---|
| HAT Assay Buffer | 25 ml | WM | BN00600-1 |
| Acetyl CoA (Lyophilized) | 1 vial | Red | BN00600-2 |
| H3 Peptide (Lyophilized) | 1 vial | Brown | BN00600-3 |
| Substrate Mix (Lyophilized) | 1 vial | Green | BN00600-4 |
| Developer | 100 µl | Orange | BN00600-5 |
| HAT Probe | 200 µl | Blue | BN00600-6 |
| CoA Standard (Lyophilized) | 1 vial | Yellow | BN00600-7 |
| Positive Control (HeLa Nuclear Extract) | 40 µl | Violet | BN00600-8 |

## V. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom. White plates are preferred for this assay.
- Multi-well spectrophotometer capable of fluorescence detection

## VI. Storage and Handling:

Store kit at -80°C, protected from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the experiment.

## VII. Reagent Preparation and Storage Conditions:

- **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.
- **H3 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.
- **Substrate Mix:** Reconstitute with 1.1 ml HAT Assay Buffer. Pipette up and down to dissolve completely. Store at -80°C. Use within two months.
- **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.
- **HAT Probe:** Warm to room temperature and mix well before use. Store at –20°C.
- **CoA Standard:** Reconstitute with 100 µl HAT Assay Buffer to generate 100 mM solution & mix completely. Aliquot and store at -80°C. Avoid repeated freeze/thaw. Use within two months.
- **Positive Control:** Aliquot & store at -80°C. Avoid repeated freeze/thaw. Use within two months.

## VIII. HAT Activity Assay Protocol:

1. **Sample Preparation:** Prepare nuclear extract using Assay Genie's Nuclear/Cytosol Fractionation Kit. Add 2-10 µl of sample and make up the volume to 50 µl with HAT Assay Buffer. Add 50 µl HAT Assay Buffer to one of the wells as Background Control. For Positive Control, add 2-4 µl of HeLa Nuclear Extract into desired wells and make up the volume to 50 µl with HAT Assay Buffer.

   **Note:**

   a. For unkown samples, use varying sample amounts so as to obtain linear enzyme activity in the range of the Standard Curve.

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

2. **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 1000 pmol/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.

3. **Reaction Mix:** 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:

| | Reaction Mix |
|---|---|
| HAT Assay Buffer | 30 µl |
| H3 Peptide | 4 µl |
| Substrate Mix | 10 µl |
| Developer | 2 µl |
| HAT Probe | 2 µl |
| Acetyl CoA | 2 µl |

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

4. **Measurement:** Read fluorescence (Ex/Em = 535/587 nm) in kinetic mode at 25°C for 30-60 min. Choose two time points ($T_1$ & $T_2$) in the linear range of the plot and obtain the corresponding RFU for Sample ($R_{S1}$ and $R_{S2}$) and sample background ($R_{B1}$ and $R_{B2}$).

5. **Calculation:** Subtract 0 Standard reading from all Standard readings. **Note:** The CoA Standards will show some drift. Extrapolate the curve for each Standard to the Y-axis to obtain the Y-intercept. Plot the Standard Curve using the corrected intercept values. Calculate the HAT Activity of the test sample $\Delta RFU = (R_{S2} - R_{S1}) - (R_{B2} - R_{B1})$. Apply the ΔRFU to the Standard Curve to get B pmol of CoA formed during the reaction time ($\Delta T = T_2 - T_1$).

$$\textbf{Sample HAT Activity} = \frac{\mathbf{B}}{\Delta T \times V} \times \mathbf{D} = \textbf{pmol/min/ml} = \textbf{µU/ml}$$

Where: **B** = CoA amount from Standard Curve (pmol)
**ΔT** = Reaction time (min.)
**V** = Sample volume added into the reaction well (ml)
**D** = 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 will generate 1.0 µmol of CoA per min. at 25°C using kit assay conditions.

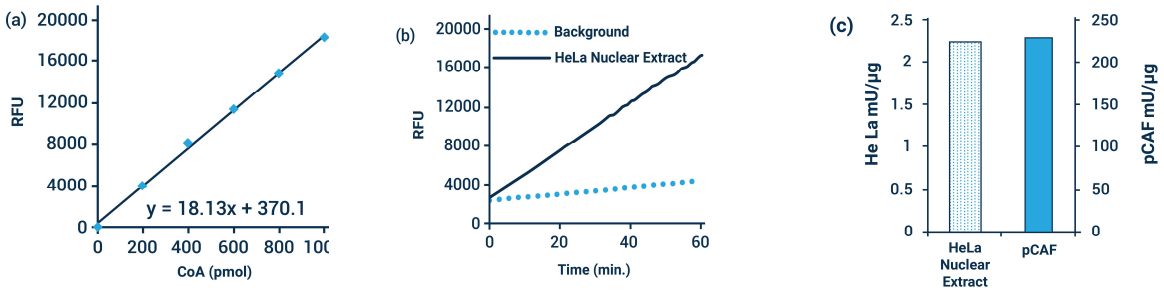


**Figure:** (a) Co A Standard Curve. (b) HAT Activity in HeLa Nuclear Extract. (c) Specific Activity of HeLa Nuclear Extract and purified recombinant pCAF. Assays were performed following the kit protocol.

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