

HAT Activity Fluorometric Assay Kit

rev. 4/14

(Catalog # K334-100; 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. BioVision'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	K334-100	Cap Code	Part Number
HAT Assay Buffer	25 ml	WM	K334-100-1
Acetyl CoA (Lyophilized)	1 vial	Red	K334-100-2
H3 Peptide (Lyophilized)	1 vial	Brown	K334-100-3
Substrate Mix (Lyophilized)	1 vial	Green	K334-100-4
Developer	100 µl	Orange	K334-100-5
PicoProbe™	200 µl	Blue	K334-100-6
CoA Standard (Lyophilized)	1 vial	Yellow	K334-100-7
Positive Control (HeLa Nuclear Extract)	40 µl	Violet	K334-100-8

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.
- **PicoProbe™:** 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 BioVision's Nuclear/Cytosol Fractionation Kit (Cat. # K266 or equivalent). 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:

- For unknown samples, use varying sample amounts so as to obtain linear enzyme activity in the range of the Standard Curve.
 - 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 μ l/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 μ l Mix containing:

	Reaction Mix
HAT Assay Buffer	30 μ l
H3 Peptide	4 μ l
Substrate Mix	10 μ l
Developer	2 μ l
PicoProbe™	2 μ l
Acetyl CoA	2 μ l

Add 50 μ l 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 Δ RFU = ($R_{S2} - R_{S1}$) - ($R_{B2} - R_{B1}$). Apply the Δ RFU to the Standard Curve to get B pmol of CoA formed during the reaction time ($\Delta T = T_2 - T_1$).

$$\text{Sample HAT Activity} = \frac{B}{\Delta T \times V} \times D = \text{pmol/min/ml} = \mu\text{U/ml}$$

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 μ U/ μ g of protein.

Unit Definition: One unit of HAT activity is the amount of enzyme that will generate 1.0 μ mol of CoA per min. at 25°C using kit assay conditions.

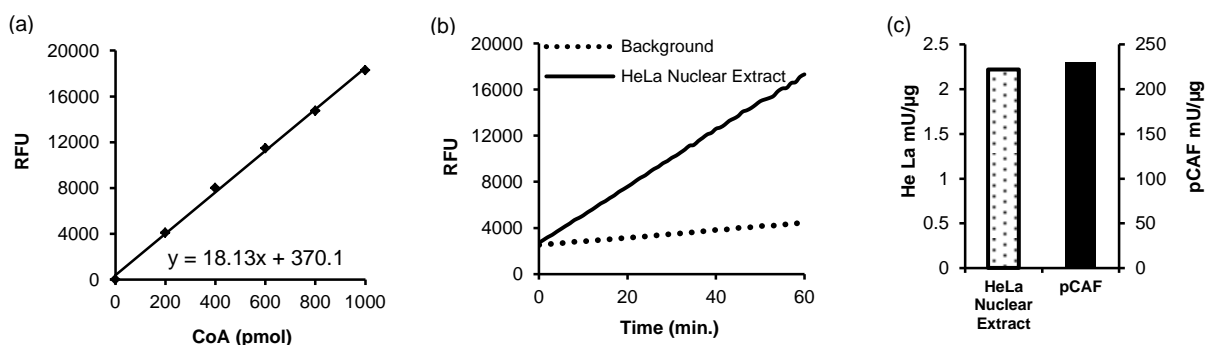


Figure: (a) Co A Standard Curve. (b) HAT Activity in HeLa Nuclear Extract. (c) Specific Activity of HeLa Nuclear Extract and purified recombinant pCAF. Assays were performed following the kit protocol.

IX. RELATED PRODUCTS:

HAT (P/CAF), human recombinant (1137)
 HAT Activator, CTPB (2086)
 HAT Activity Colorimetric Assay kit (K332)
 HAT-1 Antibody (3689)
 HAT-2 Antibody (3692)
 HAT-3 Antibody (3707)
 HAT-1 Blocking Peptide (3689BP)
 HAT-2 Blocking Peptide (3692BP)
 HAT-3 Blocking Peptide (3707BP)
 HDAC substrate, colorimetric
 Anacardic Acid (1849)
 Garcinol (2088)

HDAC Activity Colorimetric Assay kit (K331)
 HDAC Activity Fluorometric Assay kit (K330)
 HDAC Family Antibody Set (K333)
 HDAC inhibitor drug screening kit (K340)
 HDAC1 IP & Activity Assay kit (K342)
 HDAC2 IP & Activity Assay kit (K341)
 HDAC3 IP & Activity Assay kit (K344)
 HDAC3 Inhibitor screening kit (K363)
 HDAC8 Inhibitor screening kit (K368)
 Nuclear/Cytosol Fractionation Kit (k266)
 HeLa Nuclear Extract (1641)
 Plumbagin (2290)

FOR RESEARCH USE ONLY! Not to be used on humans.