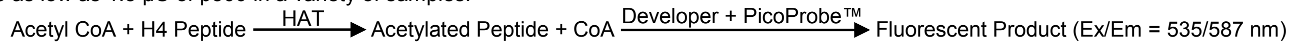
HAT (H4) Activity Fluorometric Assay Kit

(Catalog # K336-100; 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 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 PicoProbe™ 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 µU of p300 in a variety of samples.



II. Applications:

- Measurement of HAT activity of immunoprecipitated samples
- Measurement of HAT activity of purified enzyme preparations
- Measurement of HAT activity in Nuclear Extracts.

III. Sample Type:

- Immunoprecipitated HAT from cells and tissue
- Recombinant enzyme
- Nuclear Extract

IV. Kit Contents:

Components	K336-100	Cap Code	Part Number
HAT Assay Buffer	25 ml	WM	K336-100-1
Acetyl CoA (Lyophilized)	1 vial	Red	K336-100-2
H4 Peptide (Lyophilized)	1 vial	Brown	K336-100-3
Substrate Mix (Lyophilized)	1 vial	Green	K336-100-4
Developer	100 µl	Orange	K336-100-5
PicoProbe™	200 µl	Blue	K336-100-6
CoA Standard (Lyophilized)	1 vial	Yellow	K336-100-7
Positive Control (HeLa Nuclear Extract)	40 µl	Violet	K336-100-8

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. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

VII. Reagent Preparation and Storage Conditions:

- **HAT Assay Buffer:** Warm to room temperature before use.
- **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.
- **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.
- **Substrate Mix:** Reconstitute with 1.1 ml HAT Assay Buffer. Pipette up and down to dissolve. 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 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. Make up the volume to 50 µl with HAT Assay Buffer. 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.

   Notes:

   a. For unknown samples, we recommend using varying sample amounts such that enzyme activity is within the linear range of the Standard Curve.

b. 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.
c. We recommend using protease inhibitor cocktail (Cat. # K271 or equivalent) and protein A/G Sepharose (Cat. # 6503 or equivalent) for the immunoprecipitation.
d. Endogenous histones present in nuclear extracts will contribute to total HAT activity.

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
H4 Peptide	4 µl
Substrate Mix	10 µl
Diluted Developer	2 µl
PicoProbe™	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 30°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 linear portion of the time 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 ($\Delta T = T_2 - T_1$).

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

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 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.

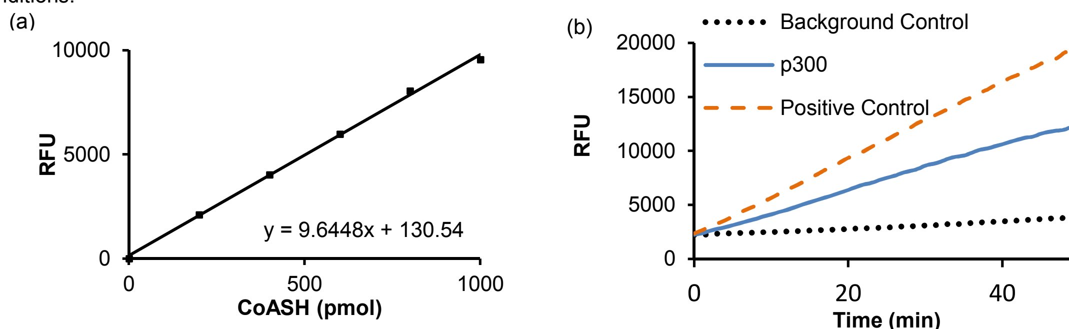


Figure: (a) CoASH Standard Curve. (b) Measurement of HAT Activity: p300 (72 ng) or HeLa nuclear extract (positive control) as per kit protocol.

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)
Plumbagin (2290)
Anacardic Acid (1849)
Garcinol (2088)
HAT Fluorometric Activity Assay Kit (K334)
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)

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