ab204709

Histone Acetyltransferase Activity Assay Kit (Fluorometric)

Instructions for Use

For rapid, sensitive and accurate detection of Histone Acetyltransferase activity.

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

Version 3 Last Updated 17 March 2022

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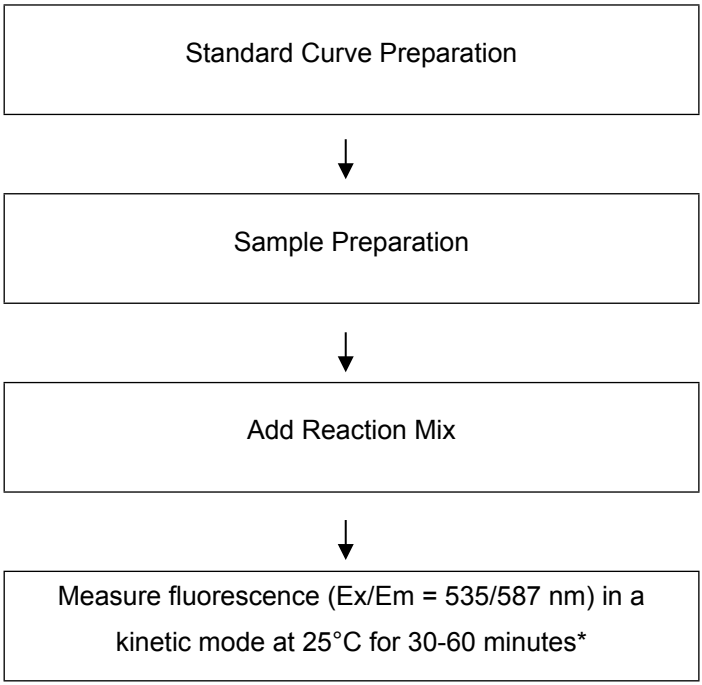
1. BACKGROUND

Histone Acetyltransferase Activity Assay Kit (Fluorometric) (ab204709) utilizes Acetyl CoA and H3 histone peptide as substrates. In this assay, HAT enzyme catalyzes the transfer of acetyl groups from AcetylCoA 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.

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.

2. ASSAY SUMMARY



**For kinetic mode detection, incubation time given in this summary is for guidance only.*

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -80ºC in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in Material Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
HAT Assay Buffer	25 mL	-80°C	-80°C
Acetyl CoA (Lyophilized)	1 Vial	-80°C	-80°C
H3 Peptide (Lyophilized)	1 Vial	-80°C	-80°C
Substrate Mix (Lyophilized)	1 Vial	-80°C	-80°C
Developer	100 µL	-80°C	-20°C
PicoProbe	200 µL	-80°C	-20°C
CoA Standard (Lyophilized)	1 Vial	-80°C	-80°C
Positive Control (HeLa Nuclear Extract)	40 µL	-80°C	-80°C

7. MATERIALS REQUIRED, NOT SUPPLIED

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

- MilliQ water or other type of double distilled water (ddH_2O)
- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader – equipped with filter Ex/Em = 535/587 nm
- 96 well plate with clear flat bottom preferably white
- Heat block or water bath
- Nuclear Extraction Kit (ab113474)

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **HAT Assay Buffer:**

Ready to use as supplied.

9.2 **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.

9.3 **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.

9.4 **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.

9.5 **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.

9.6 **PicoProbe:**

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

9.7 **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.

9.8 **Positive Control:**

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

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and cannot be stored for future use.

10.1 Prepare 1 mL of 1 mM CoA Standard by diluting 10 µL of the provided 100 mM CoA standard with 990 µL of HAT Assay Buffer.

10.2 Prepare 0.1 mL of 0.1 mM CoA Standard by diluting 10 µL of 1 mM CoA standard with 90 µL of HAT Assay Buffer.

10.3 Using 0.1 mM CoA Standard, prepare standard curve dilution as described in the table in a microplate.

Standard #	Volume of Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End Conc CoA in well (pmol/well)
1	0	150	50	0
2	6	144	50	200
3	12	138	50	400
4	18	132	50	600
5	24	126	50	800
6	30	120	50	1000

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze and store the samples immediately upon extraction at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Nuclear Extracts (from cells or tissue samples):

Prepare nuclear extracts using our Nuclear Extraction Kit (ab113474) or your preferred protocol.

Make sure samples are free of DTT or β-mercaptoethanol as these components will interfere with the assay.

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**
- **Prepare all reagents, working standards, and samples as directed in the previous sections.**

12.1 **Set up Reaction wells:**

- Standard wells = 50 µL standard dilutions.
- Sample wells = 2 – 10 µL samples (adjust volume to 50 µL/well with HAT Assay Buffer).
- Background control well= 50 µL HAT Assay Buffer).
- Positive control = 2 – 4 µL HeLa Nuclear Extract (adjust volume to 50 µL/well with HAT Assay Buffer).

12.2 **Reaction Mix:**

Prepare 50 µL of Reaction Mix for each reaction:

Component	Reaction Mix (µL)
HAT Assay Buffer	30
H3 Peptide	4
Substrate Mix	10
Developer	2
PicoProbe	2
Acetyl CoA	2

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + standards +1)

12.3 Add 50 µL of appropriate Reaction Mix into each standard, sample and positive control wells. Mix well.

12.4 Measure output on a fluorescent microplate reader at Ex/Em = 535/587 nm in a kinetic mode, every 2 – 3 minutes, for 30-60 minutes at 25°C protected from light.

NOTE: *Sample incubation time can vary depending on* Histone Acetyltransferase *(HAT) activity in the samples. We recommend measuring fluorescence in kinetic mode and then choosing two time points (T_1 and T_2) during the linear range.*

RFU value at T_2 should not exceed the highest RFU in the standard curve. For standard curve, do not subtract subtract RFU_1 from RFU_2 reading.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 If the background control is significant, then subtract the background control from sample reading.

13.3 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

NOTE: *The CoA Standards will show some drift.*

13.4 Extrapolate the curve for each Standard to the Y-axis to obtain the Y-intercept.

13.5 Plot the Standard Curve using the corrected intercept values for each standard as a function of the final concentration of CoA.

13.6 Draw the best smooth curve through these points to construct the standard curve. Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).

13.7 Activity of HAT is calculated as:

$$\Delta RFU_{535/587nm} = (RFU_{S2} - RFU_{S1}) - (RFU_{B2} - RFU_{B1})$$

Where:

RFU_{S1} is the sample reading at time T1.

RFU_{S2} is the sample reading at time T2.

RFU_{B1} is the background control sample at time T1.

RFU_{B2} is the background control sample at time T2.

Use the $\Delta RFU_{535/587nm}$ to obtain B pmol of CoA generated by Histone Acetyltransferase during the reaction time ($\Delta T = T_2 - T_1$).

13.8 Concentration of CoA in the test is calculated as:

$$HAT\ Activity = \left(\frac{B}{\Delta T x\ V}\right) * D = pmol/min/ml = \ \mu U/ml$$

Where:

B = Amount of CoA from Standard Curve (pmol).

ΔT = Reaction time (minutes).

V = Original sample volume added into the reaction well (in µL).

D = Sample dilution factor.

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

Unit Definition:

1 Unit HAT activity = amount of Histone Acetyltransferase that will generate 1.0 µmol of CoA per minutes. at 25°C using kit assay conditions.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

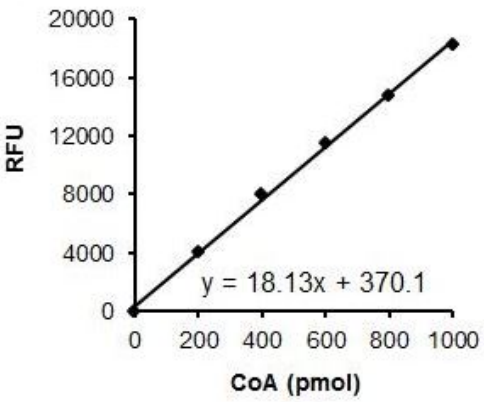


Figure 1. Typical CoA Standard calibration curve.

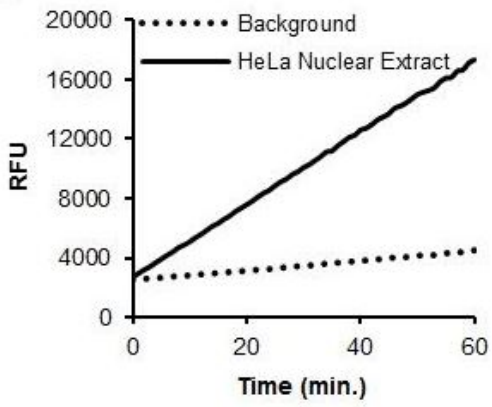


Figure 2. HAT Activity in HeLa Nuclear Extract.

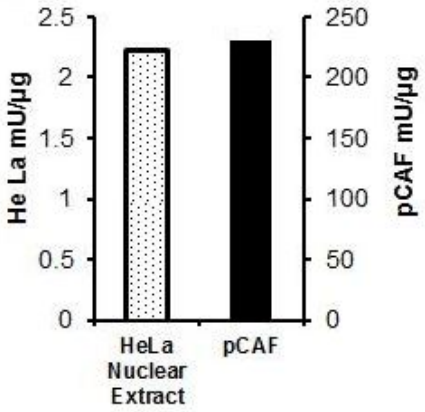


Figure 3. Specific Activity of HeLa Nuclear Extract and purified recombinant pCAF.

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare standards and prepare enzyme mix; get equipment ready.
- Prepare standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50 µL), samples (50 µL), positive control (50 µL) and background well (50 µL).
- Prepare Histone Acetyltransferase Reaction Mix (Number samples + standards + 1).

Component	Reaction Mix (µL)
HAT Assay Buffer	**30**
H3 Peptide	**4**
Substrate Mix	**10**
Developer	**2**
PicoProbe	**2**
Acetyl CoA	**2**

- Add 50 µL of Histone Acetyltransferase Reaction Mix to the standard, sample, positive control and background wells.
- Incubate plate at 25°C during 30-60 minutes and read fluorescence at Ex/Em= 535/587 nm in a kinetic mode.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17.FAQ

Which chemicals or biological materials cause interference in this assay?

Dithiothreitol (DTT) and β-mercaptoethanol will interfere with the assay.

RIPA buffer – contains SDS which can denature proteins and affect enzyme activity.

18. NOTES

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)

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