SensoLyte® HAT (pCAF) Assay Kit
Fluorimetric

Revision Number: 1.1	*Last updated: October 2014*

Catalog #	**AS-72173**
Kit Size	100 Assays (96-well plate)

- ***Optimized Performance:*** This kit is optimized to detect pCAF activity.
- ***Enhanced Value:*** It provides ample reagents to perform 100 assays in a 96-well plate format.
- ***High Speed:*** The entire process can be completed in one hour.
- ***Assured Reliability:*** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	Acetyl CoA	100 µL
Component B	Histone H3 (1-21) peptide	4 mM, 200 µL
Component C	HAT, Recombinant pCAF enzyme	20 µL, 500 ng/µL
Component D	Assay Buffer	30 mL
Component E	pCAF Developer	300 µL
Component F	Stop Solution	10 mL
Component G	CoASH standard	10 mM, 15 µL

Other Materials Required (but not provided)

- Microplate: Black, flat-bottom 96-well plate with non-binding surface.
- Fluorescence microplate reader: Capable of detecting emission at 513 nm with excitation at 389 nm.

Storage and Handling

- Store all kit components at -20°C, except for Components A and C.
- Store Components A and C at -80°C. Aliquot as needed to avoid multiple freeze-thaw cycles.
- Protect Component E from light and moisture.
- Components D and F can be stored at room temperature for convenience.

Introduction

Histone acetyltransferases (HATs) enzymes regulate the acetylation of histones and non-histone proteins.[1, 2] The acetylation of the ε-amino groups of lysine residues present at histone tails correlates largely with transcriptional activation, but it is also involved in DNA replication, DNA repair and protein–protein interactions.[3] HATs have major roles in the control of cell fate and misregulation is implicated in the development of some human tumors.[4, 5] The p300/CBP-associated factor (pCAF) acetylates specific lysines on the N-terminal tails of histones H3 and H4. The pCAF is also transcriptional coactivator for p53 factor acting as a potential tumor suppressor.[6]

The SensoLyte® HAT (pCAF) Assay Kit provides a convenient assay for screening of enzyme inhibitors and for continuous measurement of pCAF activity. After incubation with acetyl CoA and histone H3 (1-21) peptide, the pCAF enzyme generates acetylated H3 peptide and CoASH. The thiol groups of CoASH can be detected with fluorogenic reagent (ABD-F) at excitation/emission = 389nm/513nm.

Protocol

Note: For standard curve, please refer to Appendix I (optional).

1. Prepare working solutions.

Note: Thaw all kit components to room temperature before starting the experiments.

1.1 Acetyl CoA solution: Dilute Acetyl CoA (Component A) 10-fold in assay buffer (Component D) for one experiment. Refer to Table 1. For each experiment, prepare fresh solution of Acetyl CoA.

Table 1. Acetyl CoA solution for one 96-well plate (100 assays)

Components	Volume
Acetyl CoA (Component A)	100 µL
Assay buffer (Component D)	900 µL
Total volume	1mL

1.2 H3 peptide solution: Dilute H3 peptide (Component B) 10-fold in assay buffer (Component D). Refer to Table 2. This amount of peptide solution is enough for a full 96-well plate. If not using the entire plate, adjust the amount of H3 peptide to be diluted accordingly.

Table 2. H3 peptide solution for one 96-well plate (100 assays)

Components	Volume
H3 peptide (Component B)	200 µL
Assay buffer (Component D)	1800 µL
Total volume	2 mL

1.3 pCAF diluent: Dilute the pCAF enzyme (Component C) 50-fold in assay buffer (Component D). Refer to Table 3. This amount of enzyme is enough for a full 96-well plate. If not using the entire plate, adjust the amount of enzyme to be diluted accordingly.

Table 3. pCAF enzyme solution for one 96-well plate (100 assays)

Components	Volume
pCAF enzyme (Component C)	20 µL
Assay buffer (Component D)	980 µL
Total volume	1 mL

1.4 pCAF Developer solution: Dilute the pCAF Developer (Component E) 50-fold in assay buffer (Component D). Refer to Table 4.

Table 4. Developer solution for one 96-well plate (100 assays)

Components	Volume
pCAF Developer (Component E)	200 µL
Assay buffer (Component D)	9800 µL
Total volume	10 mL

2. Set up the enzymatic reaction.

2.1 Add test compounds and diluted pCAF enzyme solution to the microplate wells. For one well of a 96-well plate, the suggested volume of pCAF enzyme solution is 10 µL and 10 µL for test compound.

2.2 Simultaneously establish the following control wells, as deemed necessary:

- Positive control contains the pCAF without test compound.
- Vehicle control contains pCAF and vehicle used in delivering test compound (e.g. DMSO, concentration not to exceed 1%).
- Test compound control contains assay buffer (Component D) and test compound. Some test compounds have strong autofluorescence and may give false results.
- Background control contains the pCAF without test compounds.

2.3 Using the assay buffer (Component D), bring the total volume of all controls to 20 µL.

2.4 Pre-incubate the plate for 10 min at room temperature.

3. Run the enzymatic reaction.

3.1 Add 10 µL of Acetyl CoA solution from Step 1.1 into each well.

3.2 Add 20 µL of H3 peptide substrate solution from Step 1.2 into each well except background control wells. Mix the reagents completely by shaking the plate gently for 30 sec.

3.3 Incubate the mixture for 15 min at 37 °C and add 50 µL of Stop Solution (Component F).

Note: For best results, it is advisable to have the solutions equilibrated to the assay temperature.

3.4 Add 20 µL of H3 peptide substrate solution from Step 1.2 into background control wells.

3.5 Add 100 µL of Developer solution from Step 1.4 into each well. Incubate the reaction for 30 min at room temperature.

3.6 Measure fluorescence signal: Keep plate from direct light. Measure fluorescence intensity at Ex/Em=389/513nm. Fluorescence signal is stable at room temperature for at least 2 hours.

Appendix I. Standard Curve

- CoASH standard curve: Dilute 10 mM CoASH (Component G) 50-fold to 200 µM with assay buffer (Component D). Perform 2-fold serial dilutions to get concentrations of 100, 50, 25, 12.5, 6.25, and 3.13 µM, include an assay buffer blank. Add 50 µL/well of these serially diluted CoASH standard solutions.
- Add 50 µL/well of the Stop Solution (Component F).
- Add 100 µL/well of Developer solution from Step 1.4. Incubate the reaction for 30 min at room temperature.
- Measure the fluorescence of the CoASH standard at Ex/Em=389/513 nm. Use the same setting of sensitivity as used in the enzyme reaction.
- Plot the CoASH standard curve as RFU (relative fluorescent units) versus concentration as shown in Figure 1.
- The final concentrations of CoASH standard are 50, 25, 12.5, 6.25, 3.13, 1.57, 0.78 µM. Include an assay buffer only blank. This reference standard is used to calibrate the variation of different instruments and different experiments. It is also an indicator of the amount of final product of the enzymatic reaction.

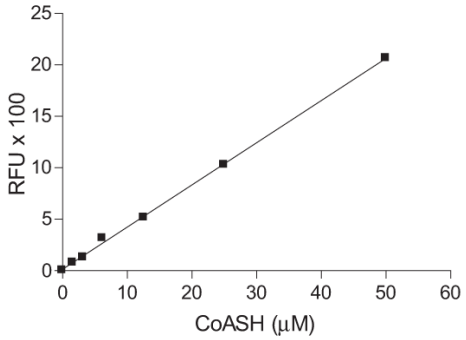

Figure 1. CoASH standard. CoASH was serially diluted in assay buffer and after 30 min incubation with Developer solution, fluorescence was recorded at Ex/Em=389/513 nm. (Flexstation 384II,Molecular Devices).

References :

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5. Iyer, NG. et al. *Oncogene.* **23,** 4225 (2004).
6. Schiltz, RL. et al. *Biochim. Biophys. Acta*. **1470,** M37 (2000).