ab115101 – Histone H3 (acetyl K9) Assay Kit (In Situ)

Instructions for Use

For the measurement of in situ acetylation of histone H3K9

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

Version 1 Last Updated 5 February 2019

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1. BACKGROUND

Acetylation of histones, including histone H3, have been involved in the regulation of chromatin structure and the recruitment of transcription factors to gene promoters. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) play a critical role in controlling histone H3 acetylation. Histone acetylation is tightly involved in cell cycle regulation, cell proliferation, and apoptosis. An imbalance in the equilibrium of histone acetylation has been associated with tumorigenesis and cancer progression. Histone H3 at lysine 9 (H3K9) acetylation is a mark of active chromatin and appears to have a dominant role in histone deposition and chromatin assembly in some organisms. Acetylation of H3K9 may be increased by inhibition of HDACs and decreased by HAT inhibition. Increased acetylation of H3K9 may also reflect a decrease in H3K9 methylation associated with gene repression.

ab115101 provides a convenient procedure to measure in situ acetylation of histone H3K9.

This kit has the following features:

- Quick and efficient procedure, which can be finished within 3 hours
- Innovative colorimetric assay without the need for radioactivity, electrophoresis, or chromatography
- Measurement of *in situ* histone H3K9 acetylation without the need to prepare cell lysates
- Microplate format makes the assay suitable for high throughput analysis of agents that increases or inhibit H3K9 acetylation
- Simple, reliable, and consistent assay conditions

Abcam's Histone H3 (acetyl K9) Assay Kit (In Situ) uses whole cell-based detection of acetylated H3K9. In this assay, adherent cells are cultured in conventional 96-well microplates. After your experimental treatment, cells are fixed and permeabilized. The acetylated H3K9 is then detected by a high affinity H3K9ac antibody. The ratio or amount of H3K9ac can be

quantified through HRP conjugated secondary antibody-color development system and is proportional to the intensity of color development.

2. ASSAY SUMMARY

Adherent cells are cultured and treated

↓

Cells are fixed, permeabilized and blocked

↓

Add capture antibody after wash

↓

Add detection antibody after wash

↓

Add developing solution for color development and measure absorbance

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 as given in the table and away from light upon receipt.

Observe the storage conditions for individual prepared components in sections 9 & 10.

For maximum recovery of the products, centrifuge the original vial prior to opening the cap.

Check if the 10X Wash Buffer and Permeabilizing Buffer contain salt precipitates before use. If so, warm at room temperature or 37°C and shake the buffer until the salts are re-dissolved.

5. MATERIALS SUPPLIED

Item	96 Tests	2 x 96 Tests	Storage Condition (Before Preparation)
10X Wash Buffer	30 mL	2 x 30 mL	4°C
Permeabilizing Buffer	30 mL	2 x 30 mL	4°C
Blocking Buffer	20 mL	2 x 20 mL	4°C
Antibody Buffer	15 mL	30 mL	4°C
Capture Antibody, 1 mg/mL	6 µL	10 µL	4°C
Detection Antibody, 400 µg/mL	20 µL	40 µL	-20°C
Developing Solution	12 mL	24 mL	4°C
Stop Solution	6 mL	12 mL	4°C
30% H_2O_2 Solution	0.5 mL	1 mL	4°C
H3K9ac Control, 20 µg/mL	15 µL	30 µL	-20°C
8-Well Control Strips	2	4	4°C
Microplates	1	2	4°C

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Orbital shaker
- Pipettes and pipette tips
- Fluorescent microplate reader
- 1.5 mL microcentrifuge tubes
- 37% Formaldehyde
- 30% H_2O_2

7. 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
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding

8. TECHNICAL HINTS

- 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
- Complete removal of all solutions and buffers during wash steps
- **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**

9. REAGENT PREPARATION

9.1 1X Wash Buffer

Dilute 10X Wash Buffer with distilled water (pH 7.2-7.5) at a 1:10 ratio (1 mL of 10X Wash Buffer + 9 mL of water).This diluted 1X Wash Buffer can now be stored at 4°C for up to six months.

9.2 1% Hydrogen Peroxide Solution

Add 330 µL of 30% H_2O_2 into 10 mL of Permeabilizing Buffer

9.3 Capture Antibody

Dilute Capture Antibody (at a 1:1000 ratio) to 1 µg/mL with Antibody Buffer.

9.4 Detection Antibody

Dilute Detection Antibody (at a 1:1000 ratio) to 0.4 µg/mL with Antibody Buffer.

10. SAMPLE PREPARATION

Inoculate and grow adherent cells in the 96-well microplate to 50-60% confluency. Leave 2-4 wells with no cell inoculation as the blank. Treat cells with the appropriate amount of reagents that may increase or reduce H3K9 acetylation for the appropriate time, based on your experiment design.

11. ASSAY PROCEDURE

11.1 Prepare fixing solution by adding 2.16 mL of 37% formaldehyde to 18 mL of PBS. Remove culture media from the wells with a quick and firm wrist-flick.

11.2 Immediately add 150 µL of fixing solution slowly to the wells and incubate at room temperature for 15 minutes. Remove fixing solution from wells with a wrist-flick. While still inverted, tap the plate gently onto absorbent paper to remove any excess fixing reagents still within the wells.

11.3 Wash the wells once (for 2 minutes) with 150 µL of the 1X Wash Buffer.

11.4 Remove the 1X Wash Buffer with a wrist flick; while still inverted, tap the plate onto absorbent paper to remove any excess solution. Add 150 µL of Permeabilizing Buffer to each well and incubate at room temperature for 5 minutes.

11.5 Remove the Permeabilizing Buffer from wells with a wrist flick. Add 100 µL of the 1% H_2O_2 Solution into each well and incubate at room temperature for 10 minutes to remove endogenous peroxidase.

11.6 Remove the 1% H_2O_2 Solution from the wells with a wrist flick and wash the wells twice with 150 µL of 1X Wash Buffer.

11.7 Remove the wash buffer with a wrist flick; while still inverted, tap the plate onto absorbent paper to remove any excess solution. Add 150 µL of Blocking Buffer to the wells and incubate at 37°C for 45 minutes. Meanwhile, add 50 µL of 1X Wash Buffer to the desired number of control strip wells, followed by adding 1 µL of H3K9ac Control at different amounts (e.g. 0.5-20 ng, diluted with distilled water) and incubate at room temperature for 30-45 minutes. For the blank wells, do not add H3K9ac Control.

11.8 Remove the Blocking Buffer with a wrist flick; while still inverted, tap the plate onto absorbent paper. Wash the wells twice with 150 µL of the 1X Wash Buffer. For each wash, remove the 1X Wash Buffer with a wrist flick; while still inverted, tap the plate onto absorbent paper. Meanwhile, aspirate the solution from control strip wells and wash the wells with 150 µL of 1X Wash Buffer three times.

11.9 Add 50 µL of the Diluted Capture Antibody to the sample wells and control strip wells. Incubate at room temperature for 60 minutes on an orbital shaker (100 rpm).

11.10 Remove solution from the wells with a wrist flick and wash the wells four times with 150 µL of 1X Wash Buffer. For each wash, remove 1X Wash Buffer with a wrist flick; while still inverted, tap the plate onto absorbent paper to remove any excess solution.

11.11 . Add 50 µL of Diluted Detection Antibody to the wells and incubate at room temperature for 30 minutes.

11.12 Remove solution from the wells with a wrist flick and wash the wells four times with 150 µL of 1X Wash Buffer. For each wash, remove 1X Wash Buffer with a wrist flick, while still inverted tap the plate onto absorbent paper to remove any excess solution.

11.13 Add 100 µL of the Developing Solution to the wells and incubate at room temperature for 1-10 minutes away from light. Monitor color development in the sample and control wells until you see a medium blue color.

11.14 Add 50 µL of Stop Solution to the wells to stop the enzyme reaction when the color in the standard wells containing the higher concentrations of standard control turns medium blue. The color should change to yellow and absorbance can be read on a microplate reader at 450 nm.

11.15 Calculate % H3K9 acetylation using the formula in Section 12 – Data Analysis.

12. ANALYSIS

Calculate the % Histone H3K9 acetylation using the following formula:

$$\text{Acetylation \%} = \frac{\text{Treated (Tested) Sample OD} - \text{Blank OD}}{\text{Untreated (Control) Sample OD} - \text{Blank OD}} \times 100\%$$

13. TROUBLESHOOTING

Problem	Cause	Solution
No Signal for Both the Positive Control and the Samples	Reagents are added incorrectly	Check if reagents are added in the proper order and if any steps of the procedure may have been omitted by mistake
	Incubation time and temperature is incorrect	Ensure the incubation time and temperature described in the protocol is followed correctly
No Signal for Only the Sample	Cells are not fixed and permeabilized sufficiently	Ensure fixation solution and permeabilizing solution are sufficiently added into the wells and incubation time is enough
High Background Present for the Blank	The well is not washed enough	Check if wash at each step is performed according to the protocol
	Overdevelopment	Decrease development time in step 11.13

14. NOTES

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