ab115104 – Histone H3 (acetyl K9) Quantification Kit (Colorimetric)

Instructions for Use

For the measurement of global histone H3K9 acetylation using a variety of mammalian cells including fresh and frozen tissues, cultured adherent and suspension cells

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

Version 1 Last Updated 1 September 2014

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1. BACKGROUND

Acetylation of histones, including histone H3, have been involved in the regulation of chromatin structure and recruitment of transcription factors to the gene promoters. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) play a critical role in the control of histone H3 acetylation at multiple sites. Histone H3 at lysine 9 (H3K9) is a primary acetylated site of histone H3. Acetylation of histone H3K9 appears to have a dominant role in histone deposition and chromatin assembly in some organisms. Acetylation of histone H3K9 is tightly involved in cell cycle regulation, cell proliferation, and apoptosis; acetylation of histone H3K9 is also an active marker. The balance between histone H3K9 acetylation and methylation is important for the establishment of specific chromatin structures. An imbalance in the equilibrium of histone H3 acetylation, including K9 acetylation, has been associated with tumorigenesis and cancer progression. Histone H3K9 acetylation may be increased by inhibition of HDACs and decreased by HAT inhibition. Thus, quantitative detection of global acetyl histone H3K9 would provide useful information for better understanding epigenetic regulation of gene activation and for developing HAT or HDAC-targeted drugs.

ab115104 provides a tool for measuring global acetylation of histone H3K9.

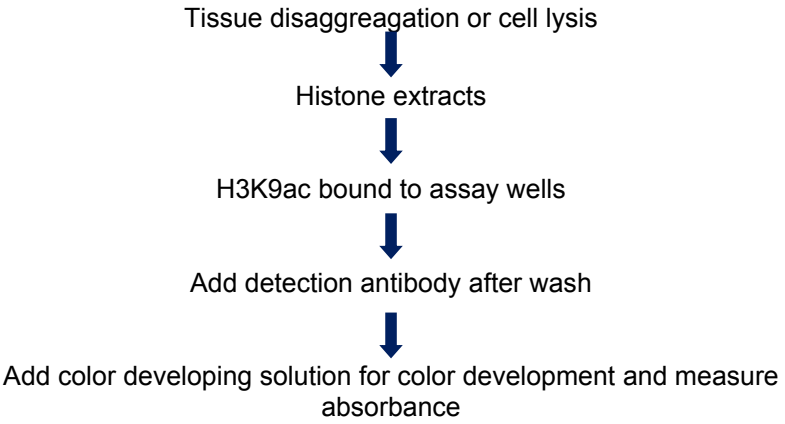
This kit has the following features:

- Quick and efficient procedure, which can be finished within 2.5 hours
- Innovative colorimetric assay without the need for radioactivity, electrophoresis, or chromatography
- Specifically captures H3K9ac with the detection limit as low as 2 ng/well and detection range from 20 ng-5 µg/well of histone extracts
- The control is conveniently included for the quantification of the amount of H3K9ac

- Strip microplate format makes the assay flexible: manual or high throughput
- Simple, reliable, and consistent assay conditions

Abcam's Histone H3 (acetyl K9) Quantification Kit (Colorimetric) is designed for measuring global histone H3K9 acetylation. In an assay with this kit, the acetyl histone H3 at lysine 9 is captured to the strip wells coated with an anti-H3K9ac antibody. The captured acetyl histone H3K9 can then be detected with a labeled detection antibody, followed by a color development reagent. The ratio of H3K9ac is proportional to the intensity of absorbance. The absolute amount of H3K9ac can be quantified by comparing to the standard control.

2. ASSAY SUMMARY



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 Antibody 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	48 Tests	96 Tests	Storage Condition (Before Preparation)
10X Wash Buffer	10 mL	20 mL	4°C
Antibody Buffer	6 mL	12 mL	4°C
Detection Antibody, 1 mg/mL	5 µL	10 µL	-20°C
Color Developer	5 mL	10 mL	4°C
Stop Solution	3 mL	6 mL	4°C
Standard Control, 100 µg/mL	10 µL	20 µL	-20°C
8-Well Sample Strips (with Frame)	4	9	4°C
8-Well Standard Control Strips*	2	3	4°C

*These wells are identified by a green ring around the top.

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Pipettes and pipette tips
- Reagent reservoirs
- Orbital shaker
- Microplate reader

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

All reagents provided are ready to use.

10. SAMPLE PREPARATION

Prepare histone extracts from cells/tissues treated or untreated by using your own successful method (acid extraction or high salt extraction). For your convenience and the best results, Abcam offers the Histone Extraction Kit (ab113476) optimized for use in Abcam's modified histone quantification series. Alternatively, preparation of histone extracts can also be performed using the procedure below:

10.1 For tissues (treated and untreated), weigh the sample and cut the sample into small pieces (1-2 mm^3) with a scalpel or scissors. Transfer tissue pieces to a Dounce homogenizer. Add TEB buffer (PBS containing 0.5% Triton X 100, 2 mM PMSF and 0.02% NaN_3) at 200 mg/mL, and disaggregate tissue pieces by 50-60 strokes. Transfer homogenized mixture to a 15 mL conical tube and centrifuge at 3000 rpm for 5 minutes at 4°C. If total mixture volume is less than 2 mL, transfer mixture to a 2 mL vial and centrifuge at 10,000 rpm for 1 minute at 4°C. Remove supernatant.

For cells (treated and untreated), harvest cells and pellet the cells by centrifugation at 1000 rpm for 5 minutes at 4°C. Resuspend cells in TEB buffer at 10^7 cells/mL and lyse cells on ice for 10 minutes with gentle stirring. Centrifuge at 3000 rpm for 5 minutes at 4°C. If total volume is less than 2 mL, transfer cell lysates to a 2 mL vial and centrifuge at 10,000 rpm for 1 minute at 4°C. Remove supernatant.

10.2 Resuspend cell/tissue pellet in 3 volumes (approx. 200 µL/10^7 cells or 200 mg tissues) of extraction buffer (0.5N HCl + 10% glycerol) and incubate on ice for 30 minutes.

10.3 Centrifuge at 12,000 rpm for 5 minutes at 4°C and remove the supernatant fraction to a new vial.

10.4 Add 8 volumes (approx. 0.6 mL/10^7 cells or 200 mg tissues) of acetone and leave at -20°C overnight.

10.5 Centrifuge at 12,000 rpm for 5 minutes and air-dry the pellet. Dissolve the pellet in distilled water (30-50 µL/10^7 cells or 200 mg tissues).

10.6 Quantify the protein concentration. Aliquot the extract and store the extract at -20°C or -80°C.

Histone extracts can be used immediately or stored at -80°C for future use.

11. PLATE PREPARATION

- Strip 1-3 (for 96 assays) or strip 1-2 (for 48 assays) – standard wells (green trimmed); the standard curve can be generated with 5-8 concentration points (includes blank).
- Example amount of standard control/well - A1: 100 ng; B1: 50 ng; C1: 25 ng; D1: 12 ng; E1: 6 ng; F1: 3 ng; G1: 1.5 ng; H1: 0 ng.
- Strip 4-12 (for 96 assays) or strip 3-6 (for 48 assays) – sample wells (No label).
- Each sample or standard point can be assayed in duplicates or triplicates.

12. ASSAY PROCEDURE

12.1 Predetermine the number of strip wells required. Remove un-needed strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C). Dilute the 10X Wash Buffer with distilled water (pH 7.2-7.5) at a 1:10 ratio to make 1X Wash Buffer (e.g. 1 mL of 10X Wash Buffer + 9 mL of water).

12.2 Add 50 µL of Antibody Buffer into each well. For the sample, add 1-2 µg of the histone extract into the sample wells. For the standard curve, dilute the Standard Control with Antibody Buffer to 1-100 ng/µL at 5-7 points (e.g., 1.5, 3, 6, 12, 25, 50, and 100 ng/µL). Add 1 µL of the Standard Control at the different concentrations into the standard wells (ringed in green). For the blank, do not add any nuclear extracts or standard control protein. Mix and cover the strip wells with Parafilm M and incubate at room temperature for 1-2 hours.

12.3 Aspirate and wash the wells with 150 µL of 1X Wash Buffer three times.

12.4 Dilute the Detection Antibody (at a 1:1000 ratio) to 1 µg/mL with Antibody Buffer. Add 50 µL of Diluted Detection Antibody to each well and incubate at room temperature for 60 minutes on an orbital shaker (100 rpm).

12.5 Aspirate and wash the wells with 150 µL of 1X Wash Buffer six times

12.6 Add 100 µL of the Color Developer into the wells and incubate at room temperature for 2-10 minutes away from light. Monitor the color development in the sample and standard wells (blue).

12.7 Add 50 µL of Stop Solution to each well to stop 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 within 2-15 minutes.

12.8 Calculate % histone H3K9 acetylation using the formulae provided in Section 13 – Data Analysis.

13. ANALYSIS

Calculate the % Histone H3K9 acetylation using the following formula:

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

For the amount quantification, plot OD versus amount of Standard Control and determine the slope as delta OD/ng.

Calculate the amount of H3K9ac using the following formula:

$$\text{Amount (ng/mg protein)} = \frac{\text{Sample OD} - \text{Blank OD}}{\text{Protein (µg)*} \times \text{Slope}} \times 1000$$

*Histone extract amount added into the sample well at step 12.2.

14. TROUBLESHOOTING

Problem	Cause	Solution
No Signal for Both the Standard Control and the Samples	Reagents are added incorrectly	Check if reagents are added in 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 are followed correctly
No Signal or Very Weak Signal for Only the Standard Control	The amount of standard control is not added into the “standard control wells,” or is added insufficiently	Ensure a sufficient amount of control is properly added to the standard control wells
No Signal for only the Sample	The protein sample is not properly extracted	Ensure the procedure and reagents are correct for the nuclear protein extraction
	The protein amount is added into well insufficiently	Ensure extract contains a sufficient amount of protein
	Protein extracts are stored incorrectly	Ensure the protein extracts are stored at -20°C or -80°C
High Background Present for the Blank	The well is not washed sufficiently	Check if wash at each step is performed according to the protocol
	Contaminated by the standard control	Ensure the well is not contaminated from adding the control protein, or from using control protein contaminated tips
	Overdevelopment	Decrease development time in step 12.6

15. NOTES

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