

# **ab115103 – Histone H4 Acetylation Assay Kit**

## Instructions for Use

For the measurement of global histone H4 acetylation using a variety of mammalian cells, including Tissue, adherent and suspension cells

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

# Table of Contents

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## INTRODUCTION

1. BACKGROUND	2
2. ASSAY SUMMARY	3

## GENERAL INFORMATION

3. PRECAUTIONS	4
4. STORAGE AND STABILITY	4
5. MATERIALS SUPPLIED	5
6. MATERIALS REQUIRED, NOT SUPPLIED	6
7. LIMITATIONS	7
8. TECHNICAL HINTS	7

## ASSAY PREPARATION

9. REAGENT PREPARATION	8
10. SAMPLE PREPARATION	8

## ASSAY PROCEDURE

11. ASSAY PROCEDURE	11
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## DATA ANALYSIS

12. ANALYSIS	12
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## RESOURCES

13. TROUBLESHOOTING	13
14. NOTES	15

## 1. BACKGROUND

Acetylation of histones, including histone H4, has 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 critical roles in controlling histone acetylation. Histone acetylation is tightly involved in cell cycle regulation, cell proliferation, and apoptosis. Reversible acetylation of nucleosomal histones H4 generally is believed to be correlated with potential transcriptional activity of eukaryotic chromatin domains. Histone H4 acetylation may be increased by inhibition of HDACs and decreased by HAT inhibition. There are very few methods used for measuring global histone H4 acetylation.

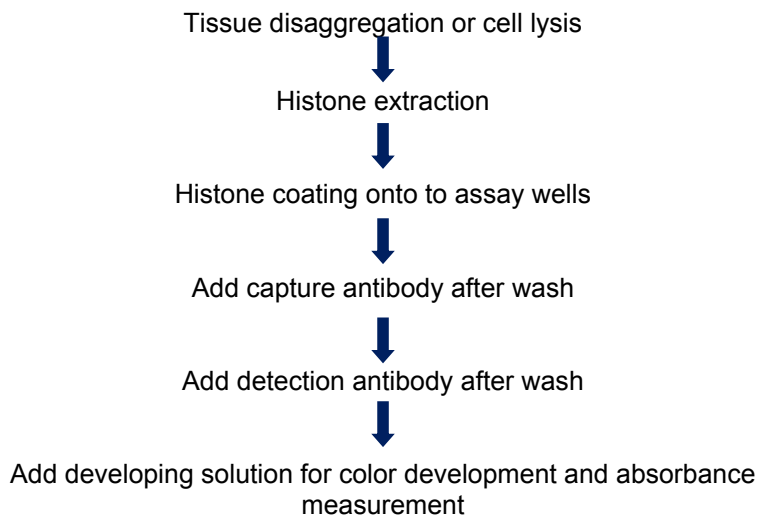
ab115103 addresses this problem by providing a unique procedure to measure global acetylation of histone H4.

This kit has the following features:

- Quick and efficient procedure, which can be finished within 5 hours.
- Innovative colorimetric assay without the need for radioactivity, electrophoresis, or chromatography.
- Strip microplate format makes the assay flexible: manual or high throughput.
- Simple, reliable, and consistent assay conditions.

The Histone H4 Acetylation Assay Kit is designed for measuring global histone H4 acetylation. In an assay with this kit, the histone proteins are stably spotted on the strip wells. The acetylated histone H4 can be recognized with a high-affinity antibody. The ratio or amount of acetylated H4 can be quantified through HRP conjugated secondary antibody-color development system and is proportional to the intensity of color development.

## 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 upon receipt and away from light.**

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 10X Wash Buffer contains 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	Quantity (48 tests)	Quantity (96 tests)	Storage Condition (Before Preparation)
10X Lysis Buffer	5 mL	10 mL	RT
Extraction Buffer	8 mL	16 mL	RT
10X Wash Buffer	14 mL	28 mL	4°C
Histone Buffer	0.5 mL	1 mL	RT
Blocking Buffer	10 mL	20 mL	4°C
Antibody Buffer	6 mL	12 mL	4°C
Capture Antibody (100 µg/mL)*	25 µL	50 µL	4°C
Detection Antibody (400 µg/mL)*	10 µL	20 µL	-20°C
Developing Solution	5 mL	10 mL	4°C
Stop Solution	3 mL	6 mL	RT
Acetylated Histone H4 Control (60 µg/mL)*	10 µL	20 µL	-20°C
8-Well Assay Strip (with Frame)	6	12	4°C

\*Spin the solution down to the bottom prior to use.

### 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
- Microplate Reader
- 1.5 mL Microcentrifuge Tubes
- 60 or 100 mm Plate
- Dounce Homogener
- 100 % TCA Solution
- Glycerol
- Acetone
- 5% HCl
- Distilled Water

### 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

Prepare fresh reagents immediately prior to use.

### 9.1 1X Lysis Buffer

Dilute 10X Lysis Buffer with distilled water (pH 7.2-7.5) at a 1:10 ratio (1 mL of 10X Lysis Buffer + 9 mL of water) to make 1X Lysis Buffer.

### 9.2 Extraction Buffer/Glycerol Solution

Add glycerol to Extraction Buffer at a 1:10 ratio (e.g. add 100  $\mu$ L of glycerol to 900  $\mu$ L of Extraction Buffer) to prepare the Extraction Buffer/Glycerol Solution.

### 9.3 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) to make 1X Wash Buffer.

### 9.4 Diluted Capture Antibody

Dilute Capture Antibody (at a 1:100 ratio) to 1  $\mu$ g/mL with Antibody Buffer.

### 9.5 Diluted Detection Antibody

Dilute Detection Antibody (at a 1:1000 ratio) to 0.4  $\mu$ g/mL with Antibody Buffer.

## 10. SAMPLE PREPARATION

### 10.1. Nucleic Extraction Protocol

10.1.1. **For tissue samples:** Place the tissue sample into a 60 or 100 mm plate. Remove unwanted tissue such as fat and necrotic material from the sample. Weigh the sample and cut the sample into small pieces (1 – 2 mm<sup>3</sup>) with a scalpel or scissors.

10.1.2. Transfer tissue pieces to a Dounce homogenizer. Add 1 mL of the 1X Lysis Buffer per every 200 mg of tissue and disaggregate tissue pieces by 10 - 30 strokes.

- 10.1.3. 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.
- 10.1.4. **For adherent cells:** Cells (treated or untreated) are grown to 70-80% confluency, then trypsinized and collected into a 15 mL conical tube. Count cells in a hemacytometer.
- 10.1.5. Centrifuge the cells at 1000 rpm for 5 minutes and discard the supernatant. Wash cells with 10 mL of PBS once by centrifugation at 1000 rpm for 5 minutes. Discard the supernatant.
- 10.1.6. Add 1X Lysis Buffer to re-suspend cell pellet (200  $\mu$ L/1 x 10<sup>6</sup> cells). Transfer cell suspension to a 1.5 mL vial and incubate on ice for 5 minutes and vortex occasionally.
- 10.1.7. Pellet cell debris by centrifuging at 12,000 rpm for 30 seconds.
- 10.1.8. **For suspension cells:** Collect cells (treated or untreated) into a 15 mL conical tube. (1 - 2 x 10<sup>6</sup> cells are required for each reaction). Count cells in a hemacytometer.
- 10.1.9. Centrifuge the cells at 1000 rpm for 5 minutes and discard the supernatant. Wash cells with 10 mL of PBS once by centrifugation at 1000 rpm for 5 minutes. Discard the supernatant.
- 10.1.10. Add 1X Lysis Buffer to re-suspend cell pellet (100  $\mu$ L/1 x 10<sup>6</sup> cells). Transfer cell suspension to a 1.5 mL vial and incubate on ice for 5 minutes and vortex occasionally.
- 10.1.11. Pellet cell debris by centrifuging at 12,000 rpm for 30 seconds.

### 10.2. Histone Extraction

- 10.2.1. Add the 1X Lysis Buffer to cell debris (10  $\mu\text{L}/1 \times 10^6$  cells or 40 mg of tissue), followed by adding 3 volumes of Extraction Buffer/Glycerol Solution. Mix by vortexing and incubate on ice for 5 minutes.
- 10.2.2. Pellet nucleic debris by centrifuging at 12,000 rpm for 5 minutes at 4°C. Transfer the supernatant to a 1.5 mL vial.
- 10.2.3. Add 100% TCA to the supernatant at a 1:4 ratio (ex: add 100  $\mu\text{L}$  of TCA to 300  $\mu\text{L}$  of supernatant. Final concentration of TCA should be 25%). Incubate on ice for 30 minutes.
- 10.2.4. Collect the precipitate by centrifuging at 12,000 rpm for 10 minutes at 4°C.
- 10.2.5. Remove supernatant and add 1 mL of acetone containing 0.1% HCl to precipitate. Mix and incubate on ice for 1 minute.
- 10.2.6. Collect the pellet by centrifuging at 12,000 rpm for 2 minutes at 4°C. Wash the pellet with 1 mL of acetone. Allow 1 minute on ice for wash.
- 10.2.7. Collect the pellet by centrifuging at 12,000 rpm for 2 minutes at 4°C. Remove the supernatant as much as possible and air dry the pellet for 5 minutes.
- 10.2.8. Add distilled water to dissolve pellet (10  $\mu\text{L}$  of water per amount of pellet extracted from  $1 \times 10^6$  cells or 40 mg of tissue) and measure histone protein concentration.

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

## 11. ASSAY PROCEDURE

- 11.1 Determine the number of strip wells required. Leave these strips in the plate frame (remaining unused strips can be placed back in the bag. Seal the bag tightly and store at 4°C).
- 11.2 Adjust protein concentration to 200 ng/μL or 400 ng/μL with Histone Buffer and add 5 μL (1 – 2 μg) of the protein solution into the central area of each well. Spread out the solution over the strip well surface by pipetting the solution up and down several times. Incubate the strip wells at 37°C (with no humidity) for 60-90 minutes to evaporate the solution and dry the wells. For the blank, add 5 μL of Histone Buffer to the wells. For the positive control, dilute Acetylated Histone H4 Control to 2-30 ng/μL with Histone Buffer and add 5 μL (10 – 150 ng) of the diluted Acetylated Histone H4 Control solution to the wells.
- 11.3 Add 150 μL of Blocking Buffer to the dried wells and incubate at 37°C for 30 minutes.
- 11.4 Aspirate and wash the wells with 150 μL of 1X Wash Buffer three times.
- 11.5 Add 50 μL of diluted Capture Antibody to the wells and incubate at room temperature for 60 minutes on an orbital shaker (50-100 rpm).
- 11.6 Aspirate and wash the wells with 150 μL of 1X Wash Buffer four times.
- 11.7 Add 50 μL of diluted Detection Buffer to the wells and incubate at room temperature for 30 minutes.
- 11.8 Aspirate and wash the wells with 150 μL of 1X Wash Buffer four times. Allow 3 minutes for the last wash.
- 11.9 Add 100 μL of Developing Solution to 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).
- 11.10 Add 50 μL of Stop Solution to the wells and read absorbance on microplate reader at 450 nm.

## 12. ANALYSIS

Calculate % histone H4 acetylation:

$$\text{Acetylation \%} = \frac{\text{Sample OD} - \text{blank OD}}{\text{Untreated control OD} - \text{blank OD}} \times 100\%$$

For the amount quantification plot OD versus amount of Acetylation Histone H4 Control and determine the slope as delta OD/ng.

Calculate the amount of acetyl H4 using the following formula:

$$\text{Amount (ng/mg protein)} = \frac{\text{OD (Sample} - \text{blank)}}{\text{Protein } (\mu\text{g})^* \times \text{Slope}} \times 1000$$

\*Histone extract amount added into the sample well at step 11.2.

## 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 order and if any steps of the procedure may have been omitted by mistake.
	The well is not completely dried.	Ensure the well is incubated without humidity and dried before adding Blocking Buffer.
	The well is incorrectly washed before protein coating.	Ensure the well is not washed before adding the positive control or protein extracts.
	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 Positive Control	The positive control is added insufficiently.	Ensure a sufficient amount of control is added to the well.
	The positive control degraded due to incorrect storage.	Follow the guidance in the protocol for storage of the positive control.

No Signal for Only the Sample	The protein sample is not properly extracted.	Follow the protocol instructions for the histone protein extraction.
	The protein amount is added into well insufficiently.	Ensure extract contains a sufficient amount of protein.
	Protein extracts are incorrectly stored or have been stored for a long period.	Ensure the protein extracts are stored at $-80^{\circ}\text{C}$ for no more than 3 months.
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 positive control.	Ensure the well is not contaminated from adding the control protein or by using control protein contaminated tips.
	Overdevelopment.	Decrease development time in 11.9.

### 14. NOTES









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