ab131562 – Histone H4 Total Acetylation Detection Fast Kit (Fluorometric)

Instructions for Use

For the measurement of total histone H4 acetylation in a fast format

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

Version 1 Last Updated 4 February 2019

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1. BACKGROUND

Acetylation of histones, including histone H4, 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 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. The reversible lysine acetylation of histone H4 may play a vital role in the regulation of many cellular processes including chromatin dynamics and transcription, gene silencing, cell cycle progression, apoptosis, differentiation, DNA replication, DNA repair, nuclear import, and neuronal repression. Detecting if histone H4 is acetylated at its lysine residue would provide useful information for further characterizing the acetylation patterns or sites, thereby leading to a better understanding of epigenetic regulation of gene activation, and development of HAT or HDAC-targeted drugs.

ab131562 provides a tool that allows the user to detect if histone H4 is acetylated and quantify the amount of the acetylated histone H4.

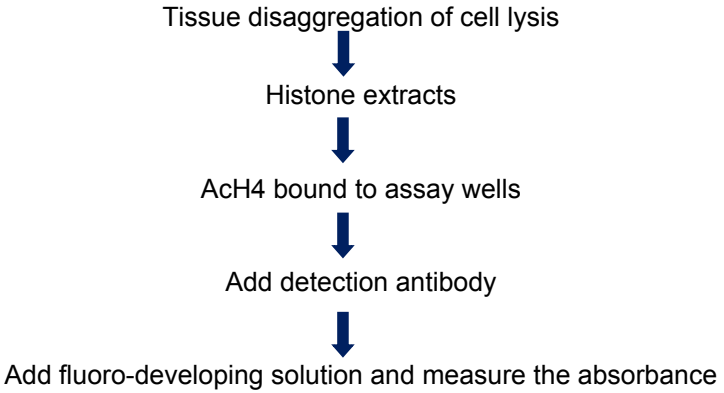
This kit has the following features:

- Quick and efficient procedure, which can be finished within 2.5 hours
- Innovative fluorometric assay without the need for radioactivity, electrophoresis, or chromatography
- Captures histone H4 acetylated at any lysine site with the detection limit as low as 0.4 ng/well and detection range from 5 ng-2 µg/well of histone extracts
- The control is conveniently included for the quantification of the amount of acetylated histone H4

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

The Histone H4 Total Acetylation Detection Fast Kit (Fluorometric) is designed for measuring total histone H4 acetylation in a fast format. In an assay with this kit, the acetyl histone H4 is captured to the strip wells which are coated with an anti-acetyl histone H4 antibody. The captured acetyl histone H4 can then be detected with a labeled detection antibody, followed by a fluoro development reagent. The ratio of acetyl histone H4 is proportional to the intensity of fluorescence. The absolute amount of acetyl histone H4 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 upon receipt 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 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	4°C
Fluoro Developer*	12 µL	24 µL	–20°C
Fluoro Enhancer*	12 µL	24 µL	4°C
Fluoro Dilution	4 mL	8 mL	4°C
Signal report solution*	5 µL	10 µL	4°C
Signal enhancer*	120 µL	240 µL	4°C
Standard Control (100 µg/mL)*	10 µL	20 µL	–20°C
8-Well Assay Strip (with Frame)	4	9	4°C
8-Well Standard Control Strips**	2	3	4°C

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

**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:

- Orbital shaker
- Pipettes and pipette tips
- Reagent reservoir
- Microplate reader
- 15 mL conical tube
- 1.5 mL microcentrifuge tubes

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 1:9 ratio (1 mL of 10X Wash Buffer to 9 mL of water).

9.2. **Fluoro-Detection Solution**

For each 1 mL of Detection solution: Add 1 µL of Detection antibody and 0.5 µL of Signal Report solution to 7 µL of 1X Wash Buffer, mix and incubate at Room Temperature (RT) for 10 minutes. Next add 20 µL of Signal Enhancer, mix and incubate at RT for 15 minutes. Finally add 970 µL of 1X Wash Buffer and Mix.

9.3. **Fluoro-Development Solution**

Adding 1 µL of Fluoro Developer and 1 µL of Fluoro Enhancer into each 400 µL of Floro Dilution.

9.4. **Standard Control**

Dilute 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).

***Note**: Keep each of diluted solutions except Diluted 1X Wash Buffer on ice until use. Any remaining diluted solutions other than Diluted 1X Wash Buffer should be discarded if not used within the same day.*

10. SAMPLE PREPARATION

10.1. 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 the modified histone quantification series. Alternatively Histone extracts can be carried out using the following method.

10.2. **Histone Extraction Protocol (Optional)**

10.2.1. **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.

10.2.2. 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.

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

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

10.2.5. 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).

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

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 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 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 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 hour.

11.3 Aspirate and wash the wells with 150 µL of 1x Wash Buffer three times.

11.4 Add 50 µL of the prepared detection solution to each well and incubate at room temperature for 60 min on an orbital shaker (100 rpm).

11.5 Aspirate and wash the wells with 150 µL of diluted 1x Wash Buffer six times.

11.6 Add 50 µL of Fluoro-Development Solution into the wells and incubate at room temperature for 1-5 minutes away from light. The color in the standard wells containing the higher concentrations may turn slightly pink during this period.

11.7 Measure and read fluorescence with a fluorescence microplate reader at 530ex/590em nm.

***Note**: If the strip well frame does not fit in the fluorescence reader, transfer the solution to a standard 96-well microplate and read fluorescence at 530ex/590em nm*

12. ANALYSIS

12.1 **Simple Calculation % Histone H4 acetylation**

$$\text{Acetylation \%} = \frac{\text{Treated (tested) Sample RFU} - \text{Blank RFU}}{\text{Untreated (control) Sample RFU} - \text{Blank RFU}} \times 100\%$$

12.2 **Calculation amount of Histone H3 acetylation**

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

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

13. TROUBLESHOOTING

Problem	Cause	Solution
No Signal for Both the Standard Control and the Samples	Reagents are added incorrectly	Check if reagents are added in order and if some steps of the procedure are omitted by mistake
	Incubation time and temperature is incorrect	Ensure the incubation time and temperature described in the protocol is 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 added to the well
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 incorrectly stored	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 by using control protein contaminated tips
	Overdevelopment	Decrease development time in step 11.6

14. NOTES

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