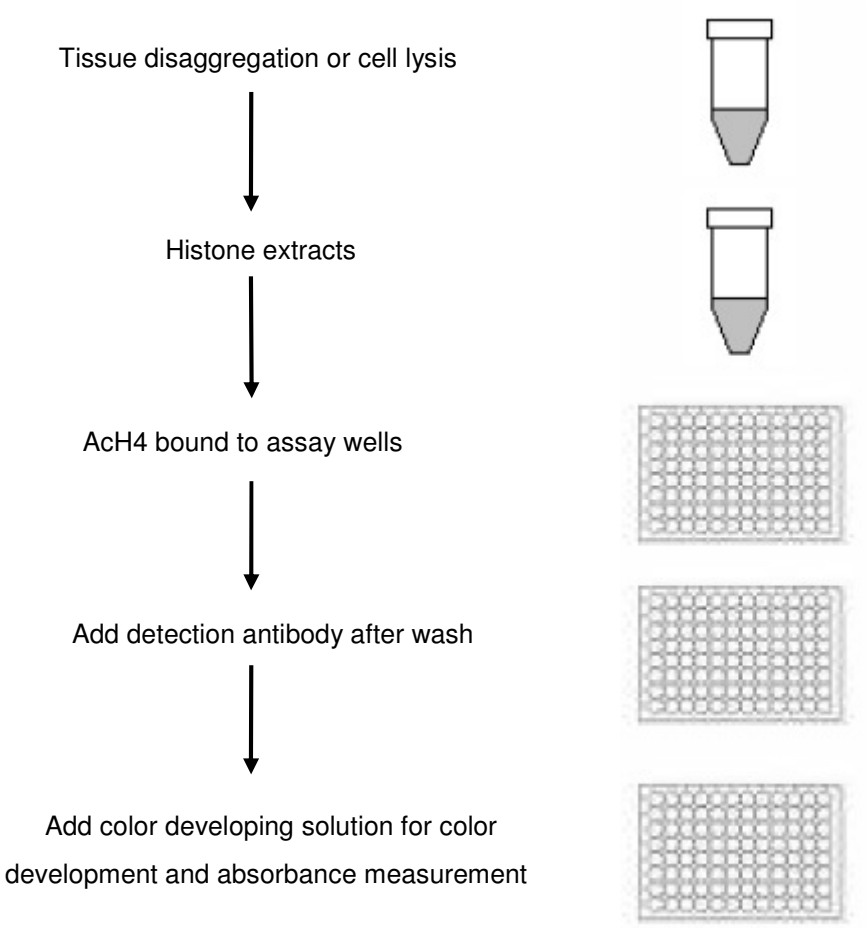
INTRODUCTION

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 acetyl transferases (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 replica-tion, 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. Total Histone H4 Acetylation Detection Fast Kit (Colorimetric) provides a tool that allows to detect if histone H4 is acetylated and quantify the amount of the acetylated histone H4. The 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.
- Captures histone H4 acetylated at any lysine site with the detection limit as low 2 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.

PRINCIPLE AND PROCEDURE

Total Histone H4 Acetylation Detection Fast Kit (Colorimetric) 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 color development reagent. The ratio of acetyl histone H4 is proportional to the intensity of absorbance. The absolute amount of acetyl histone H4 can be quantified by comparing to the standard control.



PRODUCT USE INFORMATION

Total Histone H4 Acetylation Detection Fast Kit (Colorimetric) is suitable for specifically measuring total histone H4 acetylation using a variety of mammalian cells (human, mouse, etc.) including fresh and frozen tissues, and cultured adherent and suspension cells.

Total Histone H4 Acetylation Detection Fast Kit (Colorimetric) is for research use only and is not intended for diagnostic or therapeutic application.

Suitable lab coat, disposable gloves, and eye protection are required when working with the kit.

Abnova guarantees the performance of all products in the manner described in our product instructions.

Abnova reserves the right to change or modify any product to enhance its performance and design.

Total Histone H4 Acetylation Detection Fast Kit (Colorimetric) and methods of use are covered by a pending US patent.

MATERIAL AMD METHOD

A. List of component

Components	48 assays
C1 (10X Wash Buffer)	10 ml
C2 (Antibody Buffer)	6 ml
C3 (Detection Antibody, 1 mg/ml)*	5 µl
C4 (Color Developer)	5 ml
C5 (Stop Solution)	3 ml
Signal Report Solution*	5 µl
Signal Enhancer*	120 µl
Standard Control (100 µg/ml)*	10 µl
8-Well Sample Strips (with Frame)	4
8-Well Standard Control Strips	2
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* For maximum recovery of the products, centrifuge the original vial prior to opening the cap.

SHIPPING AND STORAGE

The kit is shipped in two parts: one part at ambient room temperature, and the second part on frozen ice packs at 4℃.

Upon receipt: (1) Store C3 and Standard Control at –20℃; (2) Store all other components at 4℃ away from light. The components of the kit are stable for 6 months when stored properly.

Note: Check if buffers, C1 and C2 contain salt precipitates prior to use. If so, warm (at room temperature or 37℃) and shake the buffers until the salts are re-dissolved.

MATERIALS REQUIRED BUT NOT SUPPLIED

Orbital shaker

Pipettes and pipette tips

Reagent reservoir

Microplate reader

PROTOCOL

1. **a)** Prepare histone extracts from cells/tissues treated or untreated by using your own successful method (acid extraction or high salt extraction).

   b) Preparation of histone extracts can also be performed using the attached procedure (See Appendix). Histone extracts can be used immediately or stored at –80°C for future use.
2. 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). Dilute **C1** with distilled water (pH 7.2-7.5) at a 1: 9 ratio (ex: 1 ml of **C1**+ 9 ml of water).
3. Add 50 µl of **C2** 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 **C2** 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. 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.

   Meanwhile, prepare the Detection Solution: for every 1 ml of Detection Solution to be prepared, first add 1 µl of **C3** and 0.5 µl of Signal Report Solution to 10 µl of diluted **C1**; mix and incubate at room temperature for 10 minutes. Next, add 20 µl of the Signal Enhancer, then mix and incubate at room temperature for 15 minutes. Lastly, add 970 µl of diluted **C1** and mix.
4. Aspirate and wash each well with 150 µl of diluted C1 three times.
5. Add 50 µl of the prepared Detection Solution to each well and incubate at room temperature for 60 minutes on an orbital shaker (100 rpm).
6. Aspirate and wash each well with 150 µl of diluted **C1** six times.
7. Add 100 µl of **C4** 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 until you observe a medium blue color.
8. Add 50 µl of **C5** to each well to stop the enzymatic 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 1-15 minutes.
9. Calculate % histone H4 acetylation:

$$\text{Acetylation}\,\% = \frac{\text{OD (treated (tested) sample blank)}}{\text{OD (untreated (control) sample blank)}} \times 100\%$$

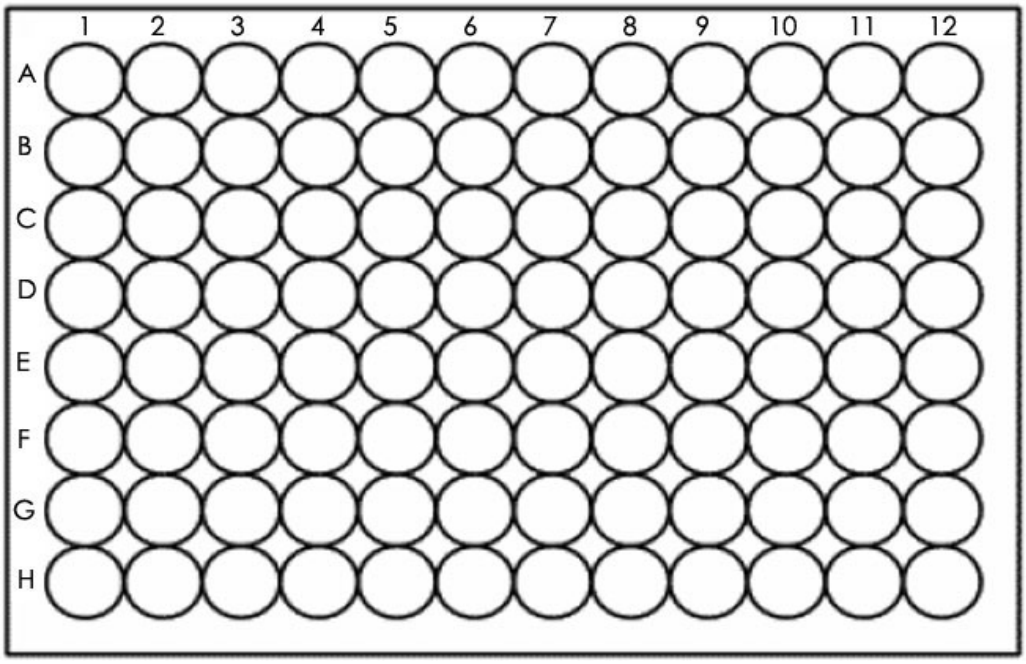
   For the amount quantification, plot OD versus amount of Standard Control and determine the slope as delta OD/ng.

   Calculate the amount of acetyl H4 using the following formula:

$$\text{Amount (ng/mg protein)} = \frac{\text{OD (sample blank)}}{\text{Protein (ug) * x slope}} \text{ x } 1000$$

   * Histone extract amount added into the sample well at step 3.

Plate configuration



- Strip 1-2 (for 48 assays): standard wells (labeld as SC); 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 3-6 (for 48 assays): sample wells (No label).
- Each sample or standard point can be assayed in the duplicates or triplicates.

HISTONE EXTRACTION PROTOCOL

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 min at 4℃. If total mixture volume is less than 2 ml, transfer mixture to a 2 ml vial and centrifuge at 10,000 rpm for 1 min at 4℃. Remove supernatant.
   For cells (treated and untreated), harvest cells and pellet the cells by centrifga-tion at 1,000 rpm for 5 min at 4℃. Resuspend cells in TEB buffer at 10^7 cells/ml and lyse cells on ice for 10 min with gentle stirring. Centrifuge at 3,000 rpm for 5 min at 4℃. If total volume is less than 2 ml, transfer cell lysates to a 2 ml vial and centrifuge at 10,000 rpm for 1 min at 4℃. Remove supernatant.
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 min.
3. Centrifuge at 12,000 rpm for 5 min at 4℃ and remove the supernatant fraction to new vial.
4. Add 8 volumes (approx. 0.6 ml/10^7 cells or 200 mg tissues) of acetone and leave overnight at –20℃.
5. Centrifuge at 12,000 rpm for 5 min and air-dry the pellet. Dissolve the pellet in distilled water (30-50 µl/10^7 cells or 200 mg tissues).
6. Quantify the protein concentration. Aliquot the extract and store the extract at –20℃ or –80℃.

TROUBLESHOOTING

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 are correctly followed.
No Signal or Very Weak Signal for Only the Standard Control	
The amount of standard control is not added into “standard control wells” or is added insufficiently.	Ensure sufficient amount of control is properly added to the standard control 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 ex-traction.
The protein amount is added into well insufficiently.	Ensure extract contains sufficient amounts of protein.
Protein extracts are incorrectly stored.	Ensure the nuclear extracts are stored at –20℃ or –80℃.
High Background Present for the Blank	
The well is not washed enough.	Check if wash at each step is per-formed according to the protocol.
Contaminated by the standard control.	Ensure the well is not contaminated by adding the control protein or by using control protein contaminated tips.
Overdevelopment.	Decrease development time in Step 7.