DATA SHEET

EpiQuik™ Total Histone H3 Acetylation Detection Fast Kit (Colorimetric) Catalog No. P-4030

INTRODUCTION

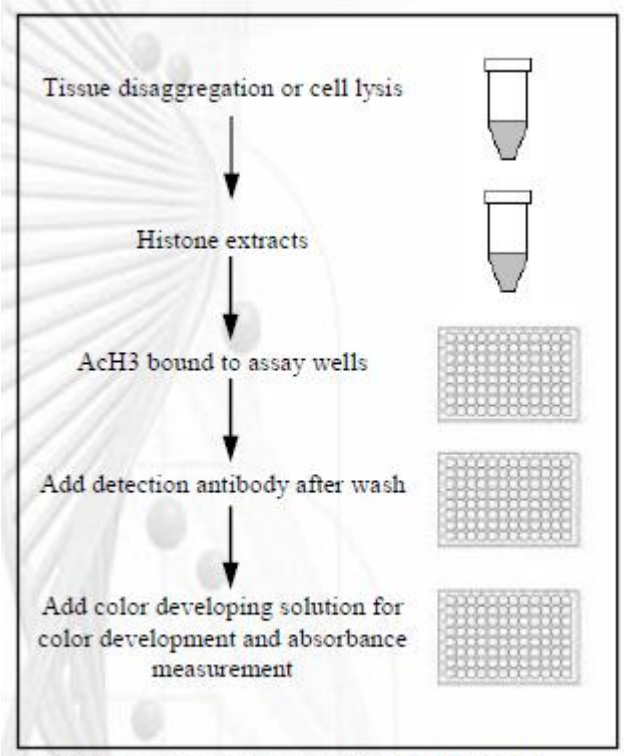
Acetylation of histones, including histone H3, have been involved in the regulation of chromatin structure and the recruitment of transcription factors to gene promoters. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) play a critical role in controlling histone H3 acetylation. Histone 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 acetylation may be increased by inhibition of HDACs and decreased by HAT inhibition. The reversible lysine acetylation of histone H3 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 H3 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. The EpiQuik™ Total Histone H3 Acetylation Detection Fast Kit (Colorimetric) provides a tool that allow to detect if histone H3 is acetylated and quantify the amount of the acetylated histone H3. 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, and chromatography.
• Captures histone H3 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 H3.
• Strip microplate format makes the assay flexible: manual or high throughput.
• Simple, reliable, and consistent assay conditions.

PRINCIPLE AND PROCEDURE

The EpiQuik™ Total Histone H3 Acetylation Detection Fast Kit (Colorimetric) is designed for measuring total histone H3 acetylation in a fast format. In an assay with this kit, the acetyl histone H3 is captured to the strip wells coated with an anti-acetyl histone H3 antibody. The captured acetyl histone H3 can then be detected with a labeled detection antibody, followed

by a color development reagent. The ratio of acetyl histone H3 is proportional to the intensity of absorbance. The absolute amount of acetyl histone H3 can be quantified by comparing to the standard control.



Schematic Procedure for Using the EpiQuik™ Total Histone H3 Acetylation Detection Fast Kit (Colorimetric)

PRODUCT USE INFORMATION

The EpiQuik™ Total Histone H3 Acetylation Detection Fast Kit (Colorimetric) is suitable for specifically measuring total histone H3 acetylation using a variety of mammalian cells (human, mouse, etc.) including fresh and frozen tissues, cultured adherent and suspension cells. The EpiQuik™ Total Histone H3 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. Epigentek guarantees the performance of all products in the manner described in our product instructions. Epigentek reserves the right to change or modify any product to enhance its performance and design. EpiQuik™ is a trademark of Epigentek Group Inc. The EpiQuik™ Total Histone H3 Acetylation Detection Fast Kit (Colorimetric) and methods of use are covered by a pending US patent.

KIT CONTENTS

Components	48 assays P-4030-48	96 assays P-4030-96
C1 (10X Wash Buffer)	10 ml	20 ml
C2 (Antibody Buffer)	6 ml	12 ml
C3 (Detection Antibody, 1 mg/ml)*	5 µl	10 µl
C4 (Color Developer)	5 ml	10 ml
C5 (Stop Solution)	3 ml	6 ml
Standard Control (100 µg/ml)*	10 µl	20 µl
8-Well Sample Strips (with Frame)	4	9
8-Well Standard Control Strips	2	3
User Guide	1	1

** 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°C. Upon receipt: (1) Store C3 and the Standard Control at –20°C; (2) Store all other components at 4°C away from light. The kit is stable for up to 6 months from the shipment date, when stored properly.

Note: Check if buffers, C1 and C2, contain salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffers until the salts are redissolved.

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) For your convenience and the best results, Epigentek offers the EpiQuik™ Total Histone Extraction Kit (Cat. No. OP-0006) optimized for use in the EpiQuik™ modified histone quantification series.
c) 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.

4. Aspirate and wash the wells with 150 µl of diluted C1 three times.

5. Dilute C3 (at a 1:1000 ratio) to 1 µg/ml with C2. Add 50 µl of diluted C3 to each well and incubate at room temperature for 60 minutes on an orbital shaker (100 rpm).

6. Aspirate and wash the wells 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 (blue).

8. Add 50 µl of C5 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. EpiQuik™ Total Histone H3 Acetylation Detection Fast Kit (Colorimetric)

9. Calculate % histone H3 acetylation:

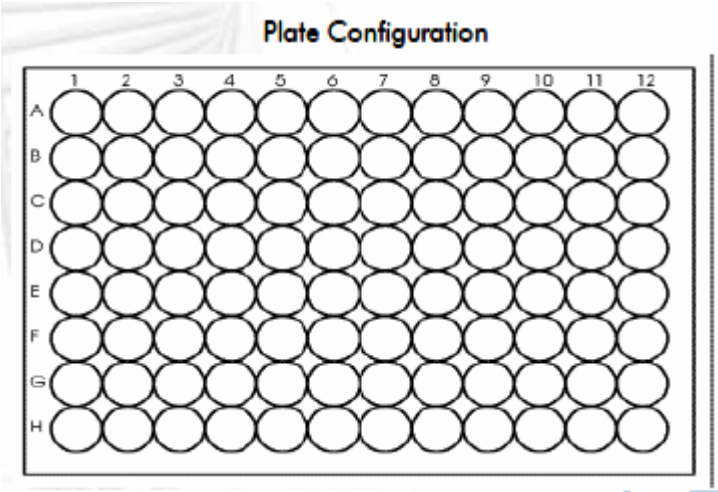
$$\text{Acetylation \%} = \frac{\text{OD (treated (}\textit{tested}\text{) sample} - \text{blank)}}{\text{OD (untreated (}\textit{control}\text{) sample} - \text{blank)}} \times 100\%$$

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

Calculate the amount of acetyl H3 using the following formula:

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

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



• Strip 1-3 (for 96 assays) or strip 1-2 (for 48 assays) - standard wells (labeled 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 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.

Appendix

Histone Extraction Protocol

1. For tissues (treated and untreated), weigh the sample and cut the sample into small pieces (1-2 mm3) 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% NaN3) 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 107 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.

2. Resuspend cell/tissue pellet in 3 volumes (approx. 200 µl/107 cells or 200 mg of tissue) of extraction buffer (0.5N HCl + 10% glycerol) and incubate on ice for 30 minutes.

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

4. Add 8 volumes (approx. 0.6 ml/107 cells or 200 mg of tissue) of acetone and leave at –20°C overnight.

5. Centrifuge at 12,000 rpm for 5 minutes and air-dry the pellet. Dissolve the pellet in distilled water (30-50 µl/107 cells or 200 mg of tissue).

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6. Quantify the protein concentration. Aliquot the extract and store the extract at –20°C or –80°C.

TROUBLESHOOTING

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 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 from using control protein contaminated tips.
Overdevelopment.	Decrease development time in Step 7.