

# EpiQuik™ Global Histone H4 Acetylation Assay Kit

Base Catalog # P-4009

## **PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE**

The *EpiQuik*™ Global Histone H4 Acetylation Assay Kit is suitable for specifically measuring global histone H4 acetylation using a variety of mammalian cells including fresh and frozen tissues, and cultured adherent and suspension cells.

## KIT CONTENTS

Components	48 assays P-4009-48	96 assays P-4009-96
GE1 (10X Lysis Buffer)	5 ml	10 ml
GE2 (Extraction Buffer)	8 ml	16 ml
GE3 (10X Wash Buffer)	14 ml	28 ml
GE4 (Histone Buffer)	0.5 ml	1 ml
GE5 (Blocking Buffer)	10 ml	20 ml
GE6 (Antibody Buffer)	6 ml	12 ml
GE7 (Capture Antibody, 100 µg/ml)*	25 µl	50 µl
GE8 (Detection Antibody, 400 µg/ml)*	10 µl	20 µl
GE9 (Developing Solution)	5 ml	10 ml
GE10 (Stop Solution)	3 ml	6 ml
Acetylated Histone H4 Control (60 µg/ml)*	10 µl	20 µl
8-Well Assay Strips (with Frame)	6	12

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

## SHIPPING & 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 **GE8** and **Acetylated Histone H4 Control** at -20°C away from light; (2) Store **GE3**, **GE5**, **GE6**, **GE7**, **GE9**, and **8-Well Assay Strips** at 4°C away from light; (3) Store **all other components** at room temperature. The components of the kit are stable for up to 6 months, when stored properly.

**Note:** Check if wash buffer, **GE3**, contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved.

## MATERIALS REQUIRED BUT NOT SUPPLIED

- 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

## GENERAL PRODUCT INFORMATION

**Usage Limitation:** The *EpiQuik*<sup>™</sup> Global Histone H4 Acetylation Assay Kit is for research use only and is not intended for diagnostic or therapeutic application.

**Safety:** Suitable lab coat, disposable gloves, and eye protection is required when working with the kit.

**Quality Control:** EpigenTek guarantees the performance of all products in the manner described in our product instructions.

**Product Updates:** EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at [www.epigentek.com/datasheet](http://www.epigentek.com/datasheet).

**Intellectual Property:** *EpiQuik*<sup>™</sup> is a trademark of EpigenTek, Inc. The *EpiQuik*<sup>™</sup> Global Histone H4 Acetylation Assay Kit and method of use contain proprietary technologies by EpigenTek.

## A BRIEF OVERVIEW

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. The *EpiQuik*<sup>™</sup> Global Histone H4 Acetylation Assay Kit addresses this problem by providing a unique procedure to measure global acetylation of histone H4. The 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.

## PRINCIPLE & PROCEDURE

The *EpiQuik*<sup>™</sup> Global 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.



Fig1. Schematic Procedure for Using the *EpiQuik*<sup>™</sup> Global Histone H4 Acetylation Assay Kit

## PROTOCOL

### Nucleic Extraction Preparation

#### For Tissue Samples:

1. 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.
2. Transfer tissue pieces to a Dounce homogenizer. Dilute **GE1** with distilled water (pH 7.2 to 7.5) at a 1:10 ratio (e.g., 1 ml of **GE1** + 9 ml of distilled water). Add 1 ml of the **diluted GE1** per every 200 mg of tissue and disaggregate tissue pieces by 10-30 strokes.

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.

For Adherent Cells:

1. 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.
2. 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.
3. Add **diluted GE1** 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.
4. Pellet cell debris by centrifuging at 12,000 rpm for 30 seconds.

For Suspension Cells:

1. 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.
2. 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.
3. Add **diluted GE1** 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.
4. Pellet cell debris by centrifuging at 12,000 rpm for 30 seconds.

### Histone Extraction

1. Add *glycerol* to **GE2** at a 1:10 ratio (e.g., add 100  $\mu$ l of *glycerol* to 900  $\mu$ l of **GE2**) to prepare the **GE2/Glycerol Solution**. Add the **diluted GE1** to cell debris (10  $\mu$ l/1 x 10<sup>6</sup> cells or 40 mg of tissue), followed by adding 3 volumes of **GE2/Glycerol Solution**. Mix by vortexing and incubate on ice for 5 minutes.
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.
3. Add 100% TCA to the supernatant at a 1:4 ratio (ex: add 100  $\mu$ l of TCA to 300  $\mu$ l of supernatant. Final concentration of TCA should be 25%). Incubate on ice for 30 minutes.
4. Collect the precipitate by centrifuging at 12,000 rpm for 10 minutes at 4°C.
5. Remove supernatant and add 1 ml of *acetone* containing 0.1% HCl to precipitate. Mix and incubate on ice for 1 minute.
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.
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.
8. Add distilled water to dissolve pellet (10  $\mu$ l of water per amount of pellet extracted from 1 x 10<sup>6</sup> cells or 40 mg of tissue) and measure histone protein concentration. The histone extract can be used immediately or stored at -80°C.

### Histone H4 Acetylation Detection

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). Dilute **GE3** with distilled water (pH 7.2-7.5) at a 1:10 ratio.
2. Adjust protein concentration to 200 ng/μl or 400 ng/μl with **GE4** 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 **GE4** to the wells. For the positive control, dilute **Acetylated Histone H4 Control** to 2-30 ng/μl with **GE4** and add 5 μl (10-150 ng) of the **diluted Acetylated Histone H4 Control** solution to the wells.
3. Add 150 μl of **GE5** to the dried wells and incubate at 37°C for 30 minutes.
4. Aspirate and wash the wells with 150 μl of **diluted GE3** three times.
5. Dilute **GE7** (at a 1:100 ratio) to 1 μg/ml with **GE6**. Add 50 μl of **diluted GE7** to the wells and incubate at room temperature for 60 minutes on an orbital shaker (50-100 rpm).
6. Aspirate and wash the wells with 150 μl of **diluted GE3** four times.
7. Dilute **GE8** (at a 1:1000 ratio) to 0.4 μg/ml with **GE6**. Add 50 μl of **diluted GE8** to the wells and incubate at room temperature for 30 minutes.
8. Aspirate and wash the wells with 150 μl of **diluted GE3** four times. Allow 3 minutes for the last wash.
9. Add 100 μl of **GE9** 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).
10. Add 50 μl of **GE10** to the wells and read absorbance on microplate reader at 450 nm.
11. Calculate % Histone H4 acetylation:

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

For accurate calculation, plot OD value versus amount of **Acetylation Histone H4 Control** and determine the slope as delta OD/ng.

Calculate the amount of acetylation H4 using the following formula:

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

## TROUBLESHOOTING

### 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 have been omitted.
The well is not completely dried.	Ensure the well is incubated without humidity and dried before adding <b>GE5</b> (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 are incorrect.	Ensure the incubation time & 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 Step 9 of "Histone H4 Acetylation Detection".

## RELATED PRODUCTS

P-3015	EpiQuik™ In Situ Histone H3-K4 Methylation Assay Kit
P-3016	EpiQuik™ In Situ Histone H3-K9 Methylation Assay Kit
P-3017	EpiQuik™ Global Histone H3-K4 Methylation Assay Kit
P-3018	EpiQuik™ Global Histone H3-K9 Methylation Assay Kit
P-4008	EpiQuik™ Global Histone H3 Acetylation Assay Kit