DATA SHEET

EpiQuik™ Global Histone H3 Acetylation Assay Kit
Catalog No. P-4008

INTRODUCTION

Acetylation of histones, including histone H3, 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 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. Currently, there are few methods used for measuring global histone H3 acetylation. The EpiQuik™ Global Histone H3 Acetylation Assay Kit addresses this problem by providing a unique procedure to measure global acetylation of histone H3.

The kit has the following features:
• Quick and efficient procedure, 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 AND PROCEDURE

The EpiQuik™ Global Histone H3 Acetylation Assay Kit is designed for measuring global histone H3 acetylation. In an assay with this kit, the histone proteins are stably spotted on the strip wells. The acetylated histone H3 can be recognized with a high-affinity antibody. The ratio or amount of acetylated histone H3 can be quantified through HRP conjugated secondary antibody-color development system and is proportional to the intensity of color development.

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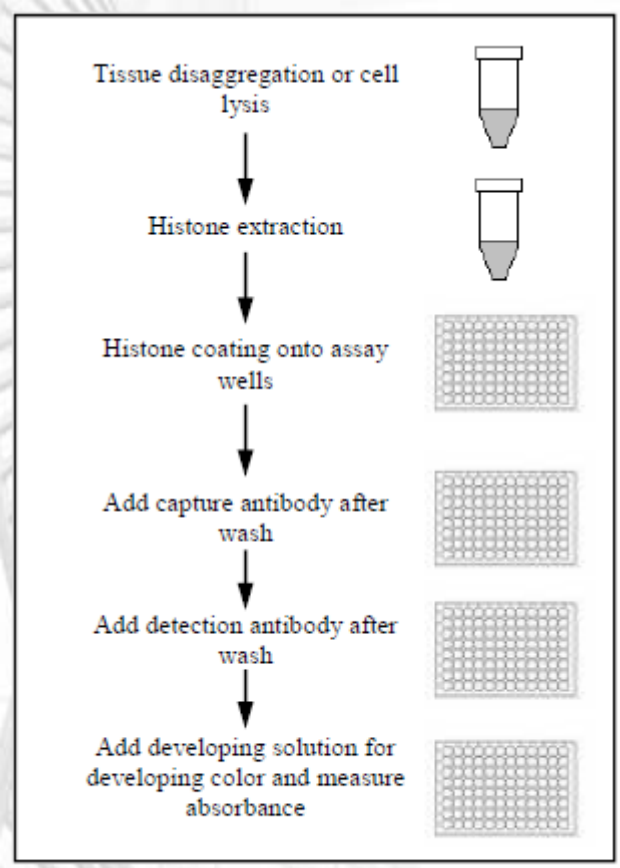

Schematic Procedure for Using the *EpiQuik™* Global Histone H3 Acetylation Assay Kit

PRODUCT USE INFORMATION

The EpiQuik™ Global Histone H3 Acetylation Assay Kit is suitable for specifically measuring global histone H3 acetylation using a variety of mammalian cells including fresh and frozen tissues, cultured adherent and suspension cells. The EpiQuik™ Global Histone H3 Acetylation Assay Kit 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, Inc. The EpiQuik™ Global Histone H3 Acetylation Assay Kit and methods of use are covered by a pending US patent.

KIT CONTENTS

Components	48 assays P-4008-48	96 assays P-4008-96
GF1 (10X Lysis Buffer)	5 ml	10 ml
GF2 (Extraction Buffer)	8 ml	16 ml
GF3 (10X Wash Buffer)	14 ml	28 ml
GF4 (Histone Buffer)	0.5 ml	1 ml
GF5 (Blocking Buffer)	10 ml	20 ml
GF6 (Antibody Buffer)	6 ml	12 ml

GF7 (Capture Antibody, 100 µg/ml)*	25 µl	50 µl
GF8 (Detection Antibody, 400 µg/ml)*	10 µl	20 µl
GF9 (Developing Solution)	5 ml	10 ml
GF10 (Stop Solution)	3 ml	6 ml
Acetylated Histone H3 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 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 Acetylated Histone H3 Control and GF8 at –20°C away from light; (2) Store GF3, GF5, GF6, GF7, GF9 and 8-Well Assay Strips at 4°C away from light; (3) Store all other components at room temperature. The kit is stable for up to 6 months from the shipment date, when stored properly.

Note: Check if wash buffer, GF3, contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are redissolved.

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

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 mm3) with a scalpel or scissors.

2. Transfer tissue pieces to a Dounce homogener. Dilute GF1 with distilled water (pH 7.2 to 7.5) at a 1:10 ratio (e.g., 1 ml of GF1 + 9 ml of distilled water). Add 1 ml of the diluted GF1 per every 200 mg of tissue and disaggregate tissue pieces with 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 the diluted GF1 to re-suspend cell pellet (200 µl/1 × 106 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 × 106 cells are required for each rxn). 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 the diluted GF1 to re-suspend cell pellet (100 µl/1 × 106 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 GF2 at a 1:10 ratio (e.g., add 100 µl of glycerol to 900 µl of GF2) to prepare GF2/Glycerol Solution. Add the diluted GF1 to cell debris (10 µl/1 × 106 cells or 40 mg tissues), followed by adding 3 volumes of GF2/Glycerol Solution. Mix by vortex 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 solution to the supernatant at a 1:4 ratio (ex: add 100 µl of TCA to 300 µl 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 µl of water per amount of pellet extracted from 1 × 106 cells or 40 mg of tissue) and measure histone protein concentration. The histone extract can be used immediately or stored at –80°C.

Histone H3 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 GF3 with distilled water (pH 7.2-7.5) at a 1:10 ratio.

2. Adjust the protein concentration to 200 ng/µl or 400 ng/µl with GF4 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 GF4 to the wells. For the positive control, dilute the Acetylated Histone H3 Control to 2-30 ng/µl with GF4. Then add 5 µl (10-150 ng) of the diluted Acetylated Histone H3 Control solution to the wells.

3. Add 150 µl of GF5 to the dried wells and incubate at 37°C for 30 minutes.

4. Aspirate and wash the wells with 150 µl of diluted GF3 three times.

5. Dilute GF7 (at a 1:100 ratio) to 1 µg/ml with GF6. Add 50 µl of diluted GF7 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 GF3 four times.

7. Dilute the GF8 (at a 1:1000 ratio) with GF6. Add 50 µl of diluted GF8 to the wells and incubate at room temperature for 30 minutes.

8. Aspirate and wash the wells with 150 µl of diluted GF3 four times. Allow 3 minutes for last wash.

9. Add 100 µl of GF9 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 GF10 to the wells and read absorbance on a microplate reader at 450 nm.

11. Calculate % histone H3 acetylation using the following formula:

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

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For accurate calculation, plot OD value versus amount of Acetylated Histone H3 Control and determine the slope as delta OD/ng. Calculate the amount of acetylation H3 using the following formula:

$$\text{Amount (ng/mg protein)} = \frac{\text{OD (sample – blank)}}{\text{slope}} \times 1000$$

TROUBLESHOOTING

No Signal for Both the Positive Control and the Samples

Reagents are added incorrectly.	Check if reagents are added in proper order and if any 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 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.

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 the 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°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 H3 Acetylation Detection."

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