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EpiQuik™ Global Histone H3-K4 Methylation Assay Kit

Base Catalog # P-3017

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik*™ Global Histone H3-K4 Methylation Assay Kit is suitable for specifically measuring global histone H3-K4 methylation using a variety of mammalian cells including fresh and frozen tissues, and cultured adherent and suspension cells.

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

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KIT CONTENTS

Components	48 assays P-3017-48	96 assays P-3017-96
GD1 (10X Lysis Buffer)	5 ml	10 ml
GD2 (Extraction Buffer)	8 ml	16 ml
GD3 (10X Wash Buffer)	14 ml	28 ml
GD4 (Histone Buffer)	0.5 ml	1 ml
GD5 (Blocking Buffer)	10 ml	20 ml
GD6 (Antibody Buffer)	6 ml	12 ml
GD7 (Capture Antibody, 100 µg/ml)*	25 µl	50 µl
GD8 (Detection Antibody, 400 µg/ml)*	10 µl	20 µl
GD9 (Developing Solution)	5 ml	10 ml
GD10 (Stop Solution)	3 ml	6 ml
Methylated H3-K4 Control (60 µg/ml)*	10 µl	20 µl
8-Well Assay Strips (with frame)	6	12
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** 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 **GD8** and **Methylated H3-K4 Control** at –20°C; (2) Store **GD3**, **GD5**, **GD7**, **GD9**, 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, **GD3**, 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*™ Global Histone H3-K4 Methylation Assay Kit is for research use only and is not intended for diagnostic or therapeutic application.

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.

Intellectual Property: The *EpiQuik*™ Global Histone H3-K4 Methylation Assay Kit and methods of use contain proprietary technologies by Epigentek. *EpiQuik*™ is a trademark of Epigentek, Inc.

A BRIEF OVERVIEW

Epigenetic activation or inactivation of genes play a critical role in many important human diseases, especially in cancer. A major mechanism for epigenetic inactivation of the genes is methylation of CpG islands in genome DNA caused by DNA methyltransferases. Histone methyltransferases (HMTs) control or regulate DNA methylation through chromatin-dependent transcription repression or activation. HMTs transfer 1-3 methyl groups from S-adenosyl-L-methionine to the lysine and arginine residues of histone proteins. SET1, SET7/9, Ash1, ALL-1, MLL, ALR, Trx, and SMYD3 are histone methyltransferases that catalyze methylation of histone H3 at lysine 4 (H3-K4) in mammalian cells. H3-K4 methylation may serve as a global epigenetic mark in euchromatin and mediates activated transcription. Increased global H3-K4 methylation is also found to be involved in some pathological processes such as cancer progress. There are only a few methods such as western blot used for measuring histone H3-K4 methylation. The methods available so far are time consuming, labor intensive, or have low throughput. The *EpiQuik*™ Global H3-K4 Methylation Assay Kit addresses these problems by using a unique procedure to measure global methylation of histone H3-K4. 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*™ Global H3-K4 Methylation Assay Kit is designed for measuring global histone H3-K4 methylation. In an assay with this kit, the histone proteins are stably spotted on the strip wells. The methylated histone H3-K4 can be recognized with a high-affinity antibody. The ratio or amount of methylated H3-K4 can be quantified through HRP conjugated secondary antibody-color development system and is proportional to the intensity of color development.

Schematic Procedure for Using the *EpiQuik*™ Global Histone H3-K4 Methylation 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 it into small pieces (1-2 mm^3) with a scalpel or scissors.
2. Transfer tissue pieces to a Dounce homogener. Dilute GD1 with distilled water at a 1:10 ratio (ex: 1 ml of GD1 + 9 ml of distilled water). Add 1 ml of the diluted GD1 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 the **diluted GD1** to re-suspend cell pellet (200 µl/1 x 10^6 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 10^6 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 10 minutes at 4°C for 5 minutes. Discard the supernatant.
3. Add the **diluted GD1** to re-suspend cell pellet (100 µl/1 x 10^6 cells). Transfer cell suspension to a 1.5 ml vial and incubate on ice for 5 minutes and vortex occasionally. Pellet cell debris by centrifuging at 12,000 rpm for 30 seconds.

Histone Extraction

1. Add *glycerol* to **GD2** at a 1:10 ratio (ex: add 100 µl of *glycerol* to 900 µl of **GD2**) to prepare the **GD2/Glycerol solution**. Add the **diluted GD1** to cell debris (10 µl/1 x 10^6 cells or 40 mg of tissue), followed by adding 3 volumes of **GD2/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 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 x 10^6 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-K4 Methylation 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 **GD3** with distilled water (pH 7.2-7.5) at a 1:10 ratio (ex: 1 ml of **GD3** + 9 ml of distilled water).
2. Adjust protein concentration to 200 ng/µl or 400 ng/µl with **GD4** and add 5 µl (1-2 µg) of the protein solution into the central area of each well. Spread out the solution over the bottom of the

strip well by pipetting the solution up and down several times, and incubate at 37°C (with no humidity) for 90 minutes to evaporate the solution and dry the wells. For the blank, add 5 µl of GD4 to the wells. For the positive control, dilute **Methylated H3-K4 Control** to 2-30 ng/µl with GD4, and then add 5 µl (10-150 ng) of the **diluted Methylated H3-K4 Control solution** to the wells.

3. Add 150 µl of GD5 to the dried wells and incubate at 37°C for 30-45 minutes.
4. Aspirate and wash the wells with 150 µl of **diluted GD3** three times.
5. Dilute GD7 (at a 1:100 ratio) to 1 µg/ml with GD6. Add 50 µl of the **diluted GD7** 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 GD3** four times.
7. Dilute GD8 (at a 1:1000 ratio) to 0.4 µg/ml with GD6. Add 50 µl of the **diluted GD8** to the wells and incubate at room temperature for 30 minutes.
8. Aspirate and wash the wells with 150 µl of the **diluted GD3** five times.
9. Add 100 µl of GD9 to the wells and incubate at room temperature for 2-10 minutes away from light. Monitor the color development in the sample and control wells (blue).
10. Add 50 µl of GD10 to the wells and read absorbance on a microplate reader at 450 nm.
11. Calculate % H3-K4 methylation:

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

For accurate calculation, plot OD value versus amount of **Methylated H3-K4 Control** and determine the slope as delta OD/ng.

Calculate the amount of methylated H3-K4 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 the proper order and if any steps of the procedure may have been omitted by mistake.
The well is not completely dried.	Ensure the well is incubated with no humidity and dry before adding the 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	Ensure the incubation time and temperature

temperature are incorrect.	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 well insufficiently.	Ensure extract contains a sufficient amount of protein.
Protein extracts are incorrectly stored or have been stored for a long time.	Ensure the protein extracts are stored at –80°C for no more than 8 weeks.

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 from using control protein contaminated tips.
Overdevelopment.	Decrease development time in Step 9 of "Histone H3-K4 Methylation Detection."

RELATED PRODUCTS

P-3002	*EpiQuik*™ Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K4)
P-3003	*EpiQuik*™ Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K9)
P-3015	*EpiQuik*™ *In Situ* Histone H3-K4 Methylation Assay Kit
P-3016	*EpiQuik*™ *In Situ* Histone H3-K9 Methylation Assay Kit
P-3018	*EpiQuik*™ Global Histone H3-K9 Methylation Assay Kit