EPIGENTEK

Version 2.0803

EpiQuik™ *In Situ* Histone H3-K9 Methylation Assay Kit

Catalog No. P-3016

User Guide*

***Always use the most updated User Guide included in your current order.**

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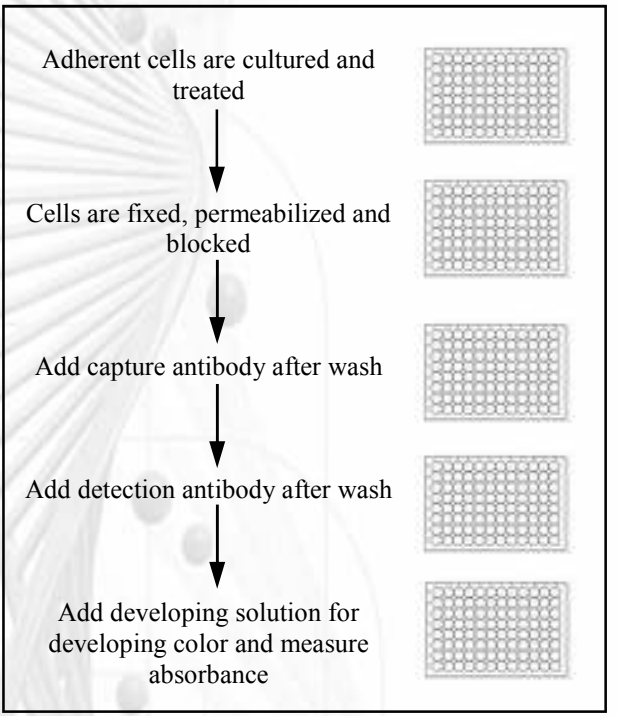
INTRODUCTION

Epigenetic activation or inactivation of genes plays 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. ESET, G9a, SUV39-h1, SUV39-h2, SETDB1, Dim-5, and Eu-HMTase are histone methyltransferases that catalyze methylation of histone H3 at lysine 9 (H3-K9) in mammalian cells. H3-K9 methylation mediates heterochromatin formation by forming a binding site for HP1 and also participates in silencing gene expression at euchromatic sites. Increased global H3-K9 methylation is also found to be involved in some pathological processes such as cancer progress. The *EpiQuik™ In Situ* Histone H3-K9 Methylation Assay Kit provides a useful tool for measuring *in situ* histone H3-K9 methylation and has the following features:

- Quick and efficient procedure, which can be finished within 3 hours.
- Innovative colorimetric assay without the need for radioactivity, electrophoresis, or chromatography.
- Measurement of *in situ* histone H3-K9 methylation without the need to prepare cell lysates.
- Microplate format makes the assay suitable for high throughput analysis of agents that increases or inhibit H3-K9 methylation.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE AND PROCEDURE

The *EpiQuik™ In Situ* Histone H3-K9 Methylation Assay Kit is a whole cell-based detection of methylated H3-K9. In this assay, adherent cells are cultured in conventional 96-well microplates. After your experimental treatment, cells are fixed and permeabilized. The methylated H3-K9 is then detected by a high affinity H3-K9 antibody. The ratio or amount of methylated H3-K9 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™ In Situ* Histone H3-K9 Methylation Assay Kit

PRODUCT USE INFORMATION

The *EpiQuik™ In Situ* Histone H3-K9 Methylation Assay Kit is suitable for specifically measuring histone H3-K9 methylation *in situ* using cultured adherent cells.

The *EpiQuik™ In Situ* Histone H3-K9 Methylation Assay Kit is for research use only and is not intended for diagnostic or therapeutic application.

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.

KIT CONTENTS

Components	96 assays P-3016-096	2 x 96 assays P-3016-192
GB1 (10X Wash Buffer)	30 ml	2 x 30 ml
GB2 (Permeabilizing Buffer)	30 ml	2 x 30 ml
GB3 (Blocking Buffer)	20 ml	2 x 20 ml
GB4 (Antibody Buffer)	15 ml	2 x 15 ml
GB5 (Capture Antibody, 100 µg/ml)*	60 µl	120 µl
GB6 (Detection Antibody, 400 µg/ml)*	20 µl	40 µl
GB7 (Developing Solution)	12 ml	24 ml
GB8 (Stop Solution)	6 ml	12 ml
30% H_2O_2 Solution	0.5 ml	1 ml
Methylated H3-K9 Control (20 µg/ml)	15 µl	30 µl
8-Well Control Strips	2	4
Microplates	1	2
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 **GB6** and **Methylated H3-K9 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, **GB1** and **GB4**, contain salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffers until the salts are re-dissolved.

PROTOCOL

Before starting, perform the following:

(A) Prepare the following required solution (not included): 37% Formaldehyde.
(B) Ensure that all buffer solutions are clear in appearance. Shake or vortex if these buffers have precipitates.

1. Grow adherent cells in the 96-well microplate to 50-60% confluency. Treat cells with the appropriate amount of reagents that may increase or reduce. H3-K9 methylation for the appropriate time, based on your experiment design.
2. Prepare the **Fixing Solution** by adding 2.16 ml of *37% formaldehyde* to 18 ml of PBS. Remove culture media from the wells with a wrist-flick.
3. Immediately add 150 µl of the **Fixing Solution** slowly to the wells and incubate at room temperature for 15 minutes. Remove the **Fixing Solution** from the wells with a wrist-flick; while still inverted, tap the plate gently onto absorbent paper to remove any excess fixing reagent still within the wells.
4. Dilute **GB1** with distilled water (pH 7.2-7.5) at a 1:10 ratio (e.g., 1 ml of **GB1** + 9 ml of distilled water). Wash the wells once (for 2 minutes) with 150 µl of the **diluted GB1**.
5. Remove the wash buffer with a wrist flick; while still inverted, tap the plate onto absorbent paper to remove any excess solution. Add 150 µl of **GB2** to each well and incubate at room temperature for 5 minutes. Meanwhile, prepare **1% H_2O_2 Solution** by adding 330 µl of **30% H_2O_2 Solution** into 10 ml of **GB2**.
6. Remove **GB2** from wells with a wrist flick and add 100 µl of the **1% H_2O_2 Solution** into each well and incubate at room temperature for 10 minutes to remove endogenous peroxidase.
7. Remove the **1% H_2O_2 Solution** from the wells with a wrist flick and wash the wells twice with 150 µl of the **diluted GB1**.
8. Remove the wash buffer with a wrist flick; while still inverted, tap the plate onto absorbent paper to remove any excess solution. Add 150 µl of **GB3** to the wells and incubate at 37°C for 45 minutes. Meanwhile, add 50 µl of **diluted GB1** to the desired number of control strip wells, followed by adding 1 µl of *methylated H3-K9 control protein* at the different amounts (ex: 0.5-20 ng, diluted with distilled water) and incubate at room temperature for 30-45 minutes. For the blank wells, do not add any *methylated H3-K9 control protein*.
9. Remove **GB3** with a wrist flick; while still inverted, tap the plate onto absorbent paper to remove any excess solution. Wash the wells twice with 150 µl of **diluted GB1**.

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For each wash, remove the **diluted GB1** with a wrist flick; while still inverted, tap the plate onto absorbent paper. Meanwhile, aspirate the solution from the control strip wells and wash the wells with 150 µl **diluted GB1** three times.

10. Dilute **GB5** (at a 1:100 ratio) to 1 µg/ml with **GB4**. Add 50 µl of the **diluted GB5** to the sample wells and the **Methylated H3-K9 Control Strip Wells**. Incubate at room temperature for 60 minutes on an orbital shaker (50-100 rpm).
11. Remove solution from the wells with a wrist flick and wash the wells four times with 150 µl of **diluted GB1**. For each wash, remove the **diluted GB1** with a wrist flick; while still inverted, tap the plate onto absorbent paper.
12. Dilute the **GB6** (at a 1:1000 ratio) to 0.4 µg/ml with **GB4**. Add 50 µl of the **diluted GB6** to the wells and incubate at room temperature for 30 minutes.
13. Remove solution from the wells with a wrist flick and wash the wells four times with 150 µl of **diluted GB1**. For each wash, remove the **diluted GB1** with a wrist flick; while still inverted, tap the plate onto absorbent paper.
14. Add 100 µl of **GB7** 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 until you observe a medium blue color.
15. Add 50 µl of **GB8** to the wells and read absorbance on a microplate reader at 450 nm.
16. Calculate % H3-K9 methylation using the following formula:

$$\text{Methylation \%} = \frac{\text{O.D. (treated sample – blank)}}{\text{O.D. (untreated control – blank)}} \times 100\%$$

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 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 for Only the Sample

Cells are not fixed and permeabilized sufficiently.	Ensure fixation solution and permeabilizing solution are sufficiently added into the cells and that the incubation time is sufficient.

High Background Present for the Blank

The wells are not washed sufficiently.	Check if wash at each step is performed according to the protocol.
Overdevelopment.	Decrease development time in Step 14.

ORDERING INFORMATION

Products	Size	Cat. No.
EpiQuik™ In Situ Histone H3-K9 Methylation Assay Kit	96 assays 2x96 assays	P-3016-096 P-3016-192

Available Related Products	Cat. No.
EpiQuik™ DNA Methyltransferase Activity/Inhibition Assay Kit	P-3001
EpiQuik™ Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K4)	P-3002
EpiQuik™ Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K9)	P-3003
EpiQuik™ In Situ Histone H3-K4 Methylation Assay Kit	P-3015
EpiQuik™ Global Histone H3-K4 Methylation Assay Kit	P-3017
EpiQuik™ Global Histone H3-K9 Methylation Assay Kit	P-3018
EpiQuik™ DNA Demethylase Activity/Inhibition Assay Kit	P-3019
EpiQuik™ Global Histone H3-K27 Methylation Assay Kit	P-3020

Need more components? You can also order parts separately by calling 1-877-374-4368 or e-mailing sales@epigentek.com.

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