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Complete Solutions for Epigenetics

EpiQuik™ In Situ Histone H3-K4 Methylation Assay Kit

Base Catalog # P-3015

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik™ In Situ* Histone H3-K4 Methylation Assay Kit is suitable for specifically measuring histone H3-K4 methylation *in situ* using cultured adherent cells.

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KIT CONTENTS

Components	96 assays P-3015-096	2 x 96 assays P-3015-192
GA1 (10X Wash Buffer)	30 ml	2 x 30 ml
GA2 (Permeabilizing Buffer)	30 ml	2 x 30 ml
GA3 (Blocking Buffer)	20 ml	2 x 20 ml
GA4 (Antibody Buffer)	10 ml	20 ml
GA5 (Capture Antibody, 100 µg/ml)*	60 µl	120 µl
GA6 (Detection Antibody, 200 µg/ml)*	20 µl	40 µl
GA7 (Developing Solution)	12 ml	24 ml
GA8 (Stop Solution)	6 ml	12 ml
30% H_2O_2 Solution	0.5 ml	1 ml
Methylated H3-K4 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 & 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 **GA6** and the **Methylated H3-K4 Control** at –20°C; (2) Store **GA1**, **GA3**, **GA5**, **GA7**, and the **8-Well Control 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 buffers, **GA1** and **GA2**, contain salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffers until the salts are re-dissolved.

GENERAL PRODUCT INFORMATION

Usage Limitation: The *EpiQuik™ In Situ* 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: *EpiQuik™* is a trademark of Epigentek Group 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. The *EpiQuik™ In Situ* Histone H3-K4 Methylation Assay Kit provides a useful tool for measuring *in situ* histone H3-K4 methylation. The kit 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-K4 methylation without the need to prepare cell lysates.
- Microplate format makes the assay suitable for high throughput analysis of agents that increases or inhibits H3-K4 methylation.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The *EpiQuik™ In Situ* Histone H3-K4 Methylation Assay Kit is a whole cell-based detection method for methylated H3-K4. In this assay, adherent cells are cultured in conventional 96-well microplates. After your experimental treatment, cells are fixed and permeabilized. The methylated H3-K4 is then detected by an anti-methyl H3-K4 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.

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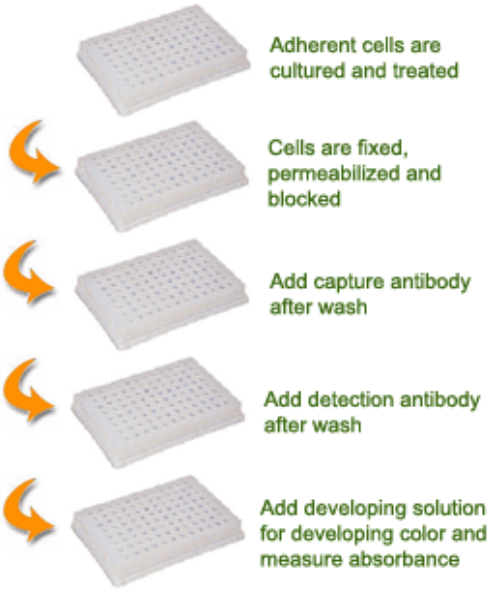

Schematic Procedure for Using the *EpiQuik™ In Situ* Histone H3-K4 Methylation Assay Kit

PROTOCOL

Before starting, perform the following:

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

1. Inoculate and grow adherent cells in the 96-well microplate to 50-70% confluency. As the blank, leave 2-4 wells without cell inoculation. Treat cells with the appropriate amount of reagents that may increase or reduce H3-K4 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 agent still within the wells.
4. Dilute **GA1** with distilled water (pH 7.2 to 7.5) at a 1:10 ratio (ex: 1 ml of **GA1** + 9 ml of distilled water) and wash wells once (2 minutes) with 150 µl of the **diluted GA1**.
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 **GA2** to each well and incubate at room temperature for 5 minutes. Meanwhile, prepare the **1% H_2O_2 solution** by adding 330 µl of *30% H_2O_2 solution* into 10 ml of **GA2**.
6. Remove **GA2** from the wells with a wrist flick. 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 **diluted GA1**.

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 **GA3** to the wells and incubate at 37°C for 45 minutes. Meanwhile, add 50 µl of **diluted GA1** to the desired number of control strip wells, followed by adding 1 µl of the **Methylated H3-K4 Control** at the different amount (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-K4 control protein.
9. Remove **GA3** with a wrist flick; while still inverted, tap the plate onto absorbent paper. Wash the wells twice with 150 µl of **diluted GA1**. For each wash, remove the **diluted GA1** 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 of **diluted GA1** three times.
10. Dilute **GA5** (at a 1:100 ratio) to 1 µg/ml with **GA4**. Add 50 µl of **diluted GA5** to the sample wells and the **Methylated H3-K4 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 GA1**. For each wash, remove the **diluted GA1** with a wrist flick; while still inverted, tap the plate onto absorbent paper.
12. Dilute **GA6** (at a 1:1000 ratio) to 0.2 µg/ml with **diluted GA4**. Add 50 µl of **diluted GA6** 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 GA1**. For each wash, remove the **diluted GA1** with a wrist flick; while still inverted, tap the plate onto absorbent paper.
14. Add 100 µl of **GA7** 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).
15. Add 50 µl of **GA8** to the wells and read absorbance on microplate reader at 450 nm.
16. Calculate % H3-K4 methylation.

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

17. Calculate methylated H3-K4 amount.

    Plot OD value versus amount of methylated H3-K4 control protein and determine the slope as delta OD/ng. Calculate methylated H3-K4 amount using the following formula:

$$\text{meH3-K4 (ng)} = \frac{\text{OD (sample – blank)}}{\text{slope}}$$

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 may have been omitted by mistake.
Incubation time and temperature are 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 cells and incubation time is adequate.
The protein amount is added into well insufficiently.	Ensure extract contains a sufficient amount of proteins.

High Background Present for the Blank

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

RELATED PRODUCTS

P-3001	*EpiQuik*™ DNA Methyltransferase Activity/Inhibition Assay Kit
P-3002	*EpiQuik*™ Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K4)
P-3003	*EpiQuik*™ Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K9)
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-3019	*EpiQuik*™ DNA Demethylase Activity/Inhibition Assay Kit
P-3020	*EpiQuik*™ Global Histone H3-K27 Methylation Assay Kit

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