EPIGENTEK

Version 2.0808

EpiQuik™ *In Situ* Histone H3-K27 Tri-Methylation Assay Kit

Catalog No. P-3014T

User Guide*

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

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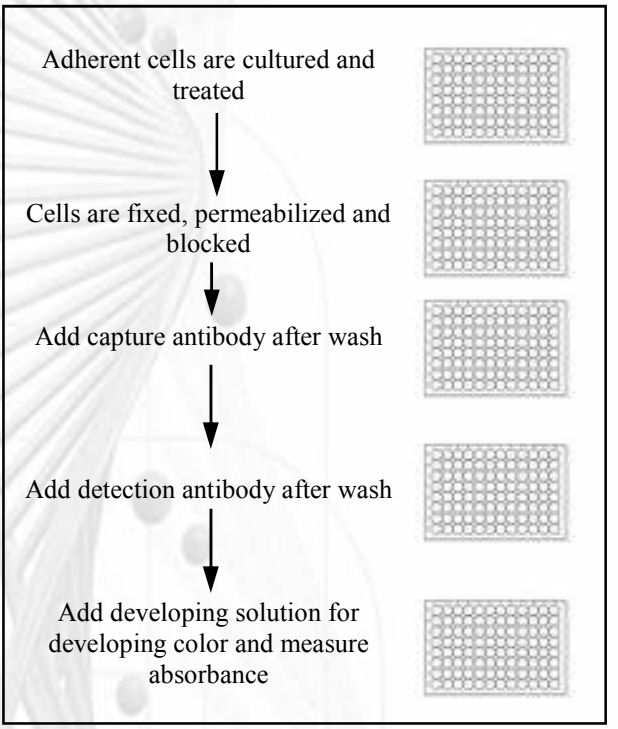
INTRODUCTION

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. G9a and polycomb group enzyme such as EZH2 are histone methyltransferases that catalyze methylation of histone H3 at lysine 27 (H3-K27) in mammalian cells. Tri-methylation of H3-K27 is a facultative heterochromatin mark, which promotes the recruitment of polycomb group proteins for gene silencing. Increased global H3-K27 tri-methylation is also found to be involved in some pathological processes such as cancer progression. There are only a couple of methods such as western blot, which are used for measuring histone H3-K27 tri-methylation. The methods available so far are time consuming and labor intensive, or have low throughput. The *EpiQuik*™ *In Situ* H3-K27 Tri-Methylation Assay Kit addresses these problems by using a unique procedure to measure *in situ* tri-methylation of histone H3-K27. 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-K27 tri-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-K27 tri-methylation.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE AND PROCEDURE

EpiQuik™ In Situ Histone H3-K27 Tri-Methylation Assay Kit is a whole cell-based detection of tri-methylated H3-K27. In this assay, adherent cells are cultured in conventional 96-well microplates. After your experimental treatment, cells are fixed and permeabilized. The tri-methylated H3-K27 is then detected by an anti-tri-methyl H3-K27 antibody. The ratio or amount of tri-methylated H3-K27 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-K27 Tri-Methylation Assay Kit

PRODUCT USE INFORMATION

The *EpiQuik™ In Situ* Histone H3-K27 Tri-Methylation Assay Kit is suitable for specifically measuring histone H3-K27 tri-methylation *in situ* using cultured adherent cells.

The *EpiQuik™ In Situ* Histone H3-K27 Tri-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 at any time to enhance its performance and design.

EpiQuik™ is a trademark of Epigentek Group Inc.

KIT CONTENTS

Components	96 assays P-3014T-096	2 x 96 assays P-3014T-192
GK1 (10X Wash Buffer)	30 ml	2 x 30 ml
GK2 (Permeabilizing Buffer)	30 ml	2 x 30 ml
GK3 (Blocking Buffer)	20 ml	2 x 20 ml
GK4 (Antibody Buffer)	10 ml	20 ml
GK5 (Capture Antibody, 1000 µg/ml)*	9 µl	18 µl
GK6 (Detection Antibody, 200 µg/ml)*	10 µl	20 µl
GK7 (Developing Solution)	12 ml	24 ml
GK8 (Stop Solution)	6 ml	12 ml
30% H_2O_2 Solution	0.5 ml	1 ml
Tri-Methyl H3-K27 Control (20 µg/ml)	15 µl	30 µl
8-Well Control Strips	2	4
Microplate	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 **GK6** and **Tri-Methyl H3-K27 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 **GK1** and **GK4** contain salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffers until the salts are re-dissolved.

Materials Required But Not Supplied

Pipettes and pipette tips
Microplate reader
15 ml conical tube
1.5 ml microcentrifuge tubes
37% formaldehyde
PBS

PROTOCOL

Before starting, perform the following:

1. Prepare the following required solutions (not included): 37% formaldehyde.
2. Ensure that all buffers are in clear solution. Shake or vortex if these buffers precipitate.

1. Inoculate and grow adherent cells in 96-well microplate to 50-70% confluency. Leave 2-4 wells with no cell inoculation as the blank. Treat cells with appropriate amount of agents that may increase or reduce H3-K27 tri-methylation for appropriate time.
2. Prepare 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 fixing solution slowly to the wells and incubate at room temperature for 15 minutes. Remove fixing solution from wells with a wrist-flick; while still inverted, tap the plate gently onto the absorbent paper to remove any excess fixing agent still within the wells.
4. Dilute **GK1** (10X Wash Buffer) with distilled water (pH 7.2 to 7.5) at a 1:10 ratio (e.g., 1 ml of **GK1** + 9 ml of distilled water). Wash wells once (for 2 minutes) with 150 µl of the **diluted GK1**.
5. Remove wash buffer with a wrist flick; while still inverted, tap the plate onto the absorbent paper to remove any excess solution. Add 150 µl of **GK2** to each well and incubate at room temperature for 5 minutes. Meanwhile, prepare *1% H_2O_2* by adding 330 µl of **30% H_2O_2 Solution** into 10 ml of **1X GK2**.
6. Remove **GK2** 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 *H_2O_2 solution* from wells with a wrist flick and wash the wells twice with 150 µl of **diluted GK1**.
8. Remove wash buffer with a wrist flick; while still inverted, tap the plate onto the absorbent paper to remove any excess solution. Add 150 µl of **GK3** to the wells and incubate at 37°C for 45 minutes. Meanwhile, add 50 µl of **diluted GK1** to the desired number of control strip wells, followed by adding 1 µl of **Tri-Methylated H3-K27 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-K4 control protein.
9. Remove **GK3** with a wrist flick; while still inverted, tap the plate onto the absorbent paper. Wash the wells twice with 150 µl of **diluted GK1**. For each wash, remove the wash buffer with a wrist flick; while still inverted, tap the plate onto the absorbent paper to remove any excess solution. Meanwhile, aspirate the solution from the control strip wells and wash the wells with 150 µl of **diluted GK1** three times.

10. Dilute **GK5** (at a 1:1000 ratio) to 1 µg/ml with **GK4**. Add 50 µl of **diluted GK5** to the sample wells and *Tri-Methyl H3-K27 control strip wells*. Incubate at room temperature for 60 minutes on an orbital shaker (100 rpm).
11. Remove solution from the wells with a wrist flick and wash the wells four times with 150 µl of **diluted GK1**. For each wash, remove the wash buffer with a wrist flick; while still inverted, tap the plate onto absorbent paper.
12. Dilute **GK6** (at a 1:1000 ratio) to 0.2 µg/ml with **1X GK4**. Add 50 µl of **diluted GK6** 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 GK1**. For each wash, remove the wash buffer with a wrist flick; while still inverted, tap the plate onto absorbent paper.
14. Add 100 µl of **GK7** 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 **GK8** to the wells and read absorbance on a microplate reader at 450 nm.
16. Calculate % H3-K27 tri-methylation:

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

17. For accurate calculation, plot OD value versus amount of **Tri-Methylated H3-K27 Control** and determine the slope as delta OD/ng.

Calculate tri-methylated H3-K27 amount using the following formula:

$$\text{Tri-Methyl H3-K27 (ng)} = \frac{\text{OD (sample – blank)}}{\text{slope}}$$

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 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 cells and incubation time is enough.
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 enough.	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-K27 Tri-Methylation Assay Kit	96 assays 2x96 assays	P-3014T-096 P-3014T-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-K9 Methylation Assay Kit	P-3016
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.