EPIGENTEK

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EpiQuik™ Global Histone H3-K27 Tri-Methylation Assay Kit

Catalog No. P-3020T

User Guide*

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

Epigentek Group Inc.
760 Parkside Avenue
Brooklyn, NY 11226
Tel: 1 877-374-4368
Fax: 1 718-484-3956
info@epigentek.com

www . EPIGENTEK . com

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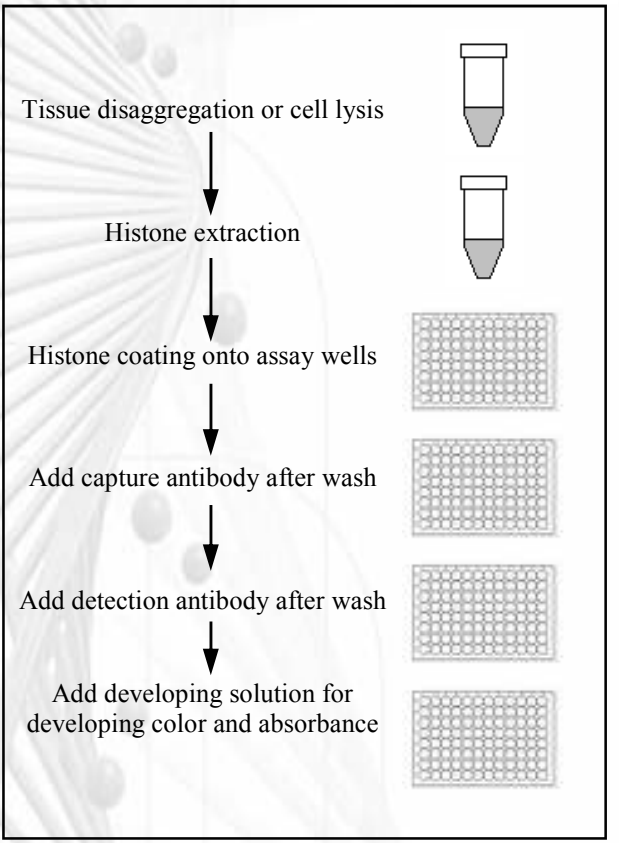
INTRODUCTION

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. 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 progress. There are only a couple of methods, such as western blot, 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*™ Global H3-K27 Tri-Methylation Assay Kit addresses these problems by using a unique procedure to measure global tri-methylation of histone H3-K27. 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 AND PROCEDURE

The *EpiQuik*™ Global H3-K27 Tri-Methylation Assay Kit is designed for measuring global histone H3-K27 tri-methylation. In an assay with this kit, the histone proteins are stably spotted on the strip wells. The tri-methylated histone H3-K27 can be recognized with a high-affinity 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*™ Global Histone H3-K27 Tri-Methylation Assay Kit

PRODUCT USE INFORMATION

The *EpiQuik*™ Global Histone H3-K27 Tri-Methylation Assay Kit is suitable for specifically measuring global histone H3-K27 tri-methylation using a variety of mammalian cells including fresh and frozen tissues, cultured adherent and suspension cells.

The *EpiQuik*™ Global Histone H3-K27 Tri-Methylation Assay Kit is for research use only and is not intended for diagnostic or therapeutic application.

Suitable lab coat, disposable gloves and eye protection is 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 Group Inc.

The *EpiQuik*™ Global Histone H3-K27 Tri-Methylation Assay Kit and methods of use are covered by a pending US patent.

KIT CONTENTS

Components	48 assays P3020T-48	96 assays P3020T-96
GT1 (10X Lysis Buffer)	5 ml	10 ml
GT2 (Extraction Buffer)	8 ml	16 ml
GT3 (10X Wash Buffer)	14 ml	28 ml
GT4 (Histone Buffer)	0.5 ml	1 ml
GT5 (Blocking Buffer)	10 ml	20 ml
GT6 (Antibody Buffer)	6 ml	12 ml
GT7 (Capture Antibody, 100 µg/ml)*	25 µl	50 µl
GT8 (Detection Antibody, 400 µg/ml)*	10 µl	20 µl
GT9 (Developing Solution)	5 ml	10 ml
GT10 (Stop Solution)	3 ml	6 ml
Tri-Methyl H3-K27 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 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 **GT8** and the **Tri-Methyl H3-K27 Control** at –20°C; (2) Store **GT3**, **GT5**, **GT7**, **GT9**, 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, **GT3**, 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

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 **GT1** with distilled water at 1:10 ratio. Add 1 ml of **diluted GT1** 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 **diluted GT1** 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 1 rpm for 10 minutes at 1000 rpm for 5 minutes. Discard the supernatant.
3. Add **diluted GT1** 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.

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Histone Extraction

1. Add *glycerol* to **GT2** at a 1:10 ratio (ex: add 100 µl of *glycerol* to 900 µl of **GT2**) to prepare the **GT2/Glycerol solution**. Add **diluted GT1** to cell debris (10 µl/1 x 10^6 cells or 40 mg of tissue), followed by adding 3 volumes of the **GT2/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% trichloroethanoic acid (TCA)* 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 x 10^6 cells or 40 mg of tissue) and measure histone protein concentration. The histone extract can be used immediately or stored at –20°C.

Histone H3-K27 Tri-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 **GT3** with distilled water (pH 7.2-7.5) at a 1:10 ratio.
2. Adjust protein concentration to 200 ng/µl or 400 ng/µl with **GT4** 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 60-90 minutes to evaporate the solution and dry the wells. For the blank, add 5 µl of **GT4** to the wells. For the positive control, dilute **Tri-Methyl H3-K27 Control** to 2-30 ng/µl with **GT4**, and then add 5 µl (10-150 ng) of the **diluted Tri-Methyl H3-K27 Control solution** to the wells.

3. Add 150 µl of **GT5** to the dried wells and incubate at 37°C for 30-45 minutes.
4. Aspirate and wash the wells with 150 µl of the **diluted GT3** three times.
5. Dilute **GT7** (at a 1:100 ratio) to 1 µg/ml with **GT6**. Add 50 µl of the **diluted GT7** 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 the **diluted GT3** four times.
7. Dilute **GT8** (at a 1:1000 ratio) to 0.4 µg/ml with **GT6**. Add 50 µl of the **diluted GT8** to the wells and incubate at room temperature for 30 minutes.
8. Aspirate and wash the wells with 150 µl of the **diluted GT3** five times.
9. Add 100 µl of **GT9** 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 **GT10** to the wells and read absorbance on microplate reader at 450 nm.
11. Calculate % H3-K27 tri-methylation:

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

For accurate calculation, plot OD value versus amount of **Tri-Methyl H3-K9 Control** and determine the slope as delta OD/ng.

Calculate the amount of tri-methylated H3-K27 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 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 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.
The positive control is degraded due to incorrect storage.	Follow the guidance in the protocol for storage of positive control.

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 were 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 enough.	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 the development time at step 9 of "Histone H3-K27 Tri-methylation Detection."

ORDERING INFORMATION

Products	Size	Cat. No.
EpiQuik™ Global Histone H3-K27 Tri-Methylation Assay Kit	48 assays	P-3020T-48
	96 assays	P-3020T-96

Available Related Products	Cat. No.
EpiQuik™ Global Histone H3-K27 Methylation Assay Kit	P-3020
EpiQuik™ *In Situ* Histone H3-K4 Methylation assay Kit	P-3015
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

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