EPIGENTEK

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# EpiQuik™ Global Tri-Methyl Histone H3-K36 Quantification Kit (Colorimetric)

Catalog No. P-3050

## User Guide*

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

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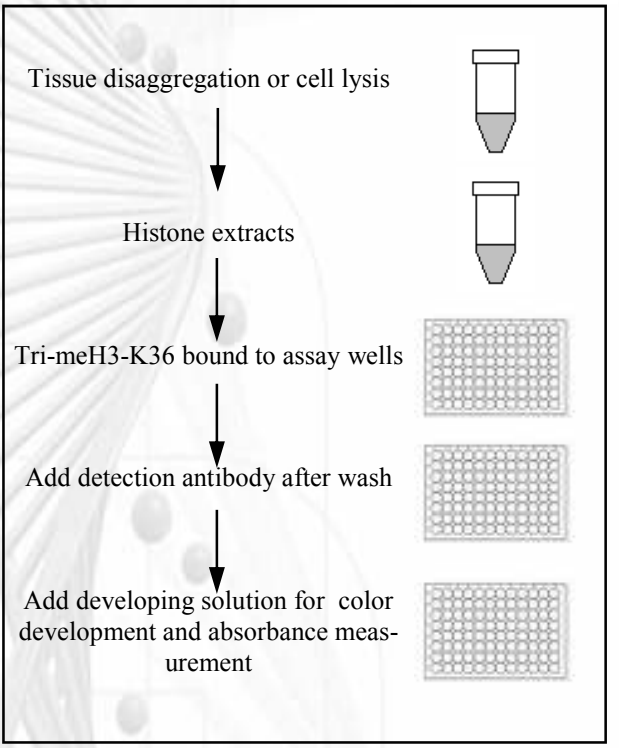
## INTRODUCTION

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. SET2 is a histone methyltransferase that catalyzes methylation of histone H3 at lysine 36 (H3-K36) in mammalian cells. H3-K36 tri-methylation is associated with transcriptionally active genes. Increased global H3-K36 methylation is also found to be linked to the Sotos syndrome and leukemia-associated protein NSD1 and the Huntington's disease protein HYPB. The global H3-K36 tri-methylation can be changed by inhibiton or activation of HMTs. Thus quantitative detection of global tri-methyl histone H3-K4 would provide useful information for better understanding epigenetic regulation of gene activation and for developing HMT-targeted drugs. The *EpiQuik*™ Global Tri-Methyl Histone H3-K36 Quantification Kit (Colorimetric) provides a tool for measuring global tri-methylation of histone H3-K36. The kit has the following features:

- Quick and efficient procedure, which can be finished within 2.5 hours.
- Innovative colorimetric assay with no need for radioactivity, electrophoresis, and chromatography.
- Specifically capturing tri-methylated H3-K36 with the detection limit as low as 2 ng/well and detection range from 20 ng-5 $\mu$g/well of histone extracts.
- The control is conveniently included for quantification of the amount of tri-methylated H3-K36.
- Strip microplate format makes the assay flexible: manual or high throughput.
- Simple, reliable, and consistent assay conditions.

## PRINCIPLE AND PROCEDURE

The *EpiQuik*™ Global Tri-Methyl Histone H3-K36 Quantification Kit (Colorimetric) is designed for measuring global histone H3-K36 tri-methylation. In an assay with this kit, the tri-methylated histone H3 at lysine 36 is captured to the strip wells coated with anti-trimethyl H3-K36 antibody. The captured tri-methylated histone H3-K36 can be then detected with a labeled detection antibody followed by color development reagent. The ratio of tri-methylated H3-K36 is proportional to the intensity of absorbance. The absolute amount of tri-methylated H3-K36 can be quantified by comparing to the standard control.




Schematic Procedure for Using the *EpiQuik*™ Global Tri-Methyl Histone H3-K36 Quantification Kit (Colorimetric)

## PRODUCT USE INFORMATION

The *EpiQuik*™ Global Tri-Methyl Histone H3-K36 Quantification Kit (Colorimetric) is suitable for specifically measuring global histone H3-K36 tri-methylation using a variety of mammalian cells (human, mouse, etc.) including fresh and frozen tissues, cultured adherent and suspension cells.

The *EpiQuik*™ Global Tri-Methyl Histone H3-K36 Quantification Kit (Colorimetric) is for research use only and is not intended for diagnostic or therapeutic application.

Suitable lab coat, disposable gloves, and eye protection are 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 Tri-Methyl Histone H3-K36 Quantification Kit (Colorimetric) and methods of use are covered by a pending US patent.

## KIT CONTENTS

| Components | 48 assays<br>P-3050-48 | 96 assays<br>P-3050-96 |
|---|---|---|
| **C1** (10X wash buffer) | 10 ml | 20 ml |
| **C2** (antibody buffer) | 6 ml | 12 ml |
| **C3** (detection antibody, 1 mg/ml)* | 5 µl | 10 µl |
| **C4** (color developer) | 5 ml | 10 ml |
| **C5** (stop solution) | 3 ml | 6 ml |
| Standard control (100 µg/ml)* | 10 µl | 20 µl |
| 8 well sample strips (with frame) | 4 | 9 |
| 8 well standard control strips | 2 | 3 |
| User guide | 1 | 1 |

** For maximum recovery of the products, centrifuge the original vial prior to opening the cap.*

## SHIPPING AND STORAGE

Upon receipt, store **C3** and **Standard control** at –20°C. Store **all other components** at 4°C away from light. The components of the kit should be stable for 6 months when stored properly.

**Note**: Check if buffers C1 and C2 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

Orbital shaker
Pipettes and pipette tips
Reagent reservoir
Microplate reader

## PROTOCOL

1. **a**) Prepare histone extracts from cells/tissues treated or untreated by using your own successful method (acid extraction or high salt extraction).
   **b**) For your convenience and best results, Epigentek offers the *EpiQuik*™ Total Histone Extraction kit (Cat # OP-0006) optimized for use in the *EpiQuik*™ modified histone quantification series.
   **c**) Preparation of histone extracts can be also performed using the attached procedure (See Appendix). Histone extracts can be used immediately or stored at –80°C for future use.

2. Determine the number of the strip wells required. Leave these strips in the plate frame (remaining unused strips can be put back in the bag. Seal the bag tightly and store at 4°C). Dilute **C1** with distilled water (pH 7.2-7.5) at the 1:9 ratios (1 ml of **C1**+ 9 ml of water).

3. Add 50 µl of **C2** into each well. For the sample, add 1-2 µg of the histone extract into the sample wells. For standard curve, dilute **standard control** with **C2** to 1 – 100 ng/µl for 5-7 points (ie: 1.5, 3, 6, 12, 25, 50, and 100 ng/µl). Add 1 µl of **standard control** at the different concentrations into the standard well. For the blank, add no nuclear extracts or no standard control protein. Mix and cover the strip wells with Parafilm M and incubate at room temperature for 60-90 min.

4. Aspirate and wash the wells with 150 µl of **diluted C1** 3 times.

5. Dilute **C3** (at 1:1000 ratio) to 1 µg/ml with **C2**. Add 50 µl of diluted **C3** to each well and incubate at room temperature for 60 min on an orbital shaker (100 rpm).

6. Aspirate and wash the wells with 150 µl of **diluted C1** 6 times.

7. Add 100 µl of **C4** into the wells and incubate at room temperature for 2-10 min away from light. Monitor color development in the sample and standard well (blue).

8. Add 50 µl of **C5** to each well to stop enzyme reaction when color in the standard wells containing the higher concentrations of standard control turns medium blue. The color should change to yellow and absorbance can be read on a microplate reader at 450 nm within 2-15 min.

9. Calculate % histone H3-K36 tri-methylation:

$$\text{Tri-methylation \%} = \frac{\text{OD (treated (tested) sample – blank)}}{\text{OD (untreated (control) sample – blank)}} \times 100\%$$

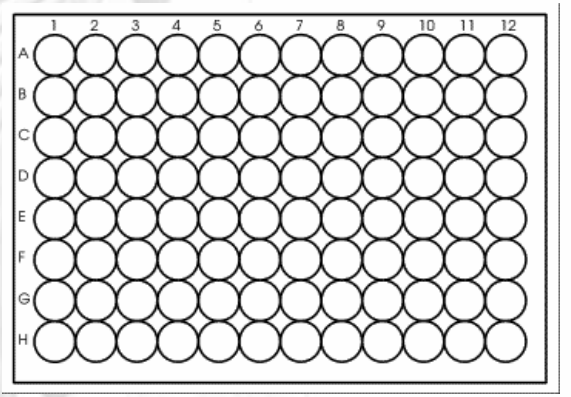
For the amount quantification, plot OD versus amount of **standard control** and determine the slope as delta OD/ng.

Calculate the amount of tri-methylated H3-K36 using the following formula:

$$\text{Amount (ng/mg protein)} = \frac{\text{OD (sample – blank)}}{\text{Protein } (\mu g)^* \text{ x slope}} \times 1000$$

* Histone extract amount added into the sample well at step 3

## Plate Configuration




- **Strip 1-3 (for 96 assays) or strip 1-2 (for 48 assays)**: standard wells (**Labeled as AC**); The standard curve can be generated with 5-8 concentration points (includes blank).
- Example amount of standard control /well: **A1**: 100 ng; **B1**: 50 ng; **C1**: 25 ng; **D1**: 12 ng; **E1**: 6 ng; **F1**: 3 ng; **G1** 1.5 ng; **H1**: 0 ng
- **Strip 4-12 (for 96 assays) or strip 3-6 (for 48 assays):** sample wells (**No label**)
- Each sample or standard point can be assayed in the duplicates or triplicates.

## APPENDIX

### Histone Extraction protocol

1. For tissues (treated and untreated). Weigh the sample and Cut the sample into small piece (1-2 $mm^3$) with a scalpel or scissors. Transfer tissue pieces to a Dounce homogener., add TEB buffer (PBS containing 0.5% Triton X 100, 2 mM PMSF and 0.02% $NaN_3$) at 200 mg/ml, and disaggregate tissue pieces by 50-60 strokes. Transfer homogenized mixture to a 15 ml conical tube and centrifuge at 3,000 rpm for 5 min 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 min at 4°C. Remove supernatant.

   For cells (treated and untreated). Harvest cells and pellet the cells by centrifgation at 1000 rpm for 5 min at 4°C. Resuspend cells in TEB buffer at $10^7$ cells/ml and lyse cells on ice for 10 min with gentle stirring. Centrifuge at 3000 rpm for 5 min at 4°C. If total volume is less than 2 ml, transfer cell lysates to a 2 ml vial and centrifuge at 10000 rpm for 1 min at 4°C. Remove supernatant.

2. Resuspend cell/tissue pellet in 3 volumes (approx. 200 $\mu l/10^7$ cells or 200 mg tissues) of extraction buffer (0.5N HCl + 10% glycerol) and incubate on ice for 30 min.

3. Centrifuge at 12,000 rpm for 5 min at 4°C and remove the supernatant fraction to new vial.

4. Add 8 volumes ( approx. 0.6 ml/ $10^7$ cells or 200 mg tissues) of acetone and leave at –20°C overnight.

5. Centrifuge at 12,000 rpm for 5 min and air-dry the pellet. Dissolve the pellet in distilled water (30-50 $\mu l/10^7$ cells or 200 mg tissues).

6. Quantify the protein concentration. Aliquot the extract and store the extract at –20°C or –80°C.

## TROUBLESHOOTING

### No Signal for Both the Standard Control and the Samples

| | |
|---|---|
| Reagents are added incorrectly. | Check if reagents are added in order and if some steps of the procedure are omitted by mistake. |
| Incubation time and temperature is incorrect. | Ensure the incubation time and temperature described in the protocol are correctly followed. |

### No Signal or Very Weak Signal for Only the Standard Control

| | |
|---|---|
| The amount of standard control is not added into "standard control wells" or is added insufficiently . | Ensure sufficient amount of control is properly added to the standard control well |

### No Signal for Only the Sample

| | |
|---|---|
| The protein sample is not properly extracted. | Ensure the procedure and reagents are correct for the nuclear protein extraction. |
| The protein amount is added into well insufficiently. | Ensure extract contains sufficient amount of proteins. |
| Protein extracts are incorrectly stored. | Ensure the nuclear extracts are stored at –20°C or –80°C. |

### High Background Present for the Blank

| | |
|---|---|
| The well is not washed enough. | Check if wash at each step is per formed according the protocol. |
| Contaminated by the standard control. | Ensure the well is not contaminated by adding the control protein or by using control protein contaminated tips. |
| Overdevelopment. | Decrease development time in Step 7 . |

## ORDERING INFORMATION

| Products | Size | Cat. No. |
|---|---|---|
| *EpiQuik*™ Global Tri-Methyl Histone H3-K36 Quantification Kit (Colorimetric) | 48 assays<br>96 assays | P-3050-48<br>P-3050-96 |

| Available Related Products | Cat. No. |
|---|---|
| *EpiQuik*™ Global Mono-Methyl Histone H3-K36 Quantification Kit (Colorimetric) | P-3046 |
| *EpiQuik*™ Global Mono-Methyl Histone H3-K36 Quantification Kit (Fluorometric) | P-3047 |
| *EpiQuik*™ Global Di-Methyl Histone H3-K36 Quantification Kit (Colorimetric) | P-3048 |
| *EpiQuik*™ Global Di-Methyl Histone H3-K36 Quantification Kit (Fluorometric) | P-3049 |
| *EpiQuik*™ Global Tri-Methyl Histone H3-K36 Quantification Kit (Fluorometric) | P-3051 |
| *EpiQuik*™ Global Pan-Methyl Histone H3-K36 Quantification Kit (Colorimetric) | P-3052 |
| *EpiQuik*™ Global Pan-Methyl Histone H3-K36 Quantification Kit (Fluorometric) | P-3053 |

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