EPIGENTEK
Complete Solutions for Epigenetics

EpiQuik™ Global Di-Methyl Histone H3-K4 Quantification Kit (Fluorometric)

Base Catalog # P-3023

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik*™ Global Di-Methyl Histone H3-K4 Quantification Kit (Fluorometric) is suitable for specifically measuring global histone H3-K4 di-methylation using a variety of mammalian cells (human, mouse, etc.) including fresh and frozen tissues, cultured adherent and suspension cells.

Suitable lab coat, disposable gloves, and eye protection are required when working with the kit.

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KIT CONTENTS

Components	48 assays P-3023-48	96 assays P-3023-96
F1 (10X Wash Buffer)	10 ml	20 ml
F2 (Antibody Buffer)	6 ml	12 ml
F3 (Detection Antibody, 1 mg/ml)*	5 µl	10 µl
F4 (Fluoro Developer)*	12 µl	24 µl
F5 (Fluoro Enhancer)*	12 µl	24 µl
F6 (Fluoro Dilution)	4 ml	8 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
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** 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 **F3**, **F4**, and the **Standard 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, **F1** and **F2**, 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
- ☐ Fluorescence microplate reader
- ☐ 15 ml conical tube
- ☐ 1.5 ml microcentrifuge tubes

GENERAL PRODUCT INFORMATION

Usage Limitation: The *EpiQuik*™ Global Di-Methyl Histone H3-K4 Quantification Kit (Fluorometric) 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: The *EpiQuik*™ Global Di-Methyl Histone H3-K4 Quantification Kit (Fluorometric) and methods of use contain proprietary technologies by Epigentek. *EpiQuik*™ is a trademark of Epigentek Group Inc.

A BRIEF OVERVIEW

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. 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 di-methylation is associated with transcriptionally permissive euchromatin regions in the genome and may serve as a global epigenetic mark in euchromatin and mediates activated transcription. Increased global H3-K4 di-methylation is also found to be involved in some pathological processes such as cancer progression. The global H3-K4 di-methylation can also be changed by inhibition or activation of HMTs. Thus, quantitative detection of global di-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 Di-Methyl Histone H3-K4 Quantification Kit (Fluorometric) provides a tool for measuring global di-methylation of histone H3-K4. The kit has the following features:

- Quick and efficient procedure, which can be finished within 2.5 hours.
- Innovative fluorometric assay without need for radioactivity, electrophoresis, or chromatography.
- Specifically captures di-methylated H3-K4 with the detection limit as low as 0.2 ng/well and detection range from 20 ng-5 μg/well of histone extracts.
- The control is conveniently included for the quantification of the amount of di-methylated H3-K4.
- Strip microplate format makes the assay flexible: manual or high throughput.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The *EpiQuik*™ Global Di-Methyl Histone H3-K4 Quantification Kit (Fluorometric) is designed for measuring global histone H3-K4 di-methylation. In an assay with this kit, the di-methylated histone H3 at lysine 4 is captured to the strip wells coated with an anti-di-methyl H3-K4 antibody. The

captured di-methylated histone H3-K4 can then be detected with a labeled detection antibody, followed by a fluorescent development reagent. The ratio of di-methylated H3-K4 is proportional to the intensity of fluorescence. The absolute amount of di-methylated H3-K4 can be quantitated by comparing to the standard control

Schematic Procedure for Using the *EpiQuik*™ Global Di-Methyl Histone H3-K4 Quantification Kit (Fluorometric)

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 the best results, Epigentek offers the *EpiQuik*™ Total Histone Extraction Kit (Cat. No. OP-0006) optimized for use in the *EpiQuik*™ modified histone quantification series.
   c. Preparation of histone extracts can also be 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 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 **F1** with distilled water (pH 7.2-7.5) at a 1:9 ratio (ex: 1 ml of **F1** + 9 ml of distilled water).

3. Add 50 µl of **F2** into each well. For the sample, add 50-200 ng of the histone extract into the sample wells. For the standard curve, dilute the **Standard Control** with **F2** to 1 – 100 ng/µl at 5-7 points (e.g., 1.5, 3, 6, 12, 25, 50, and 100 ng/µl). Add 1 µl of the **Standard Control** at the different concentrations into the standard wells. For the blank, do not add any nuclear extracts or standard control protein. Mix and cover the strip wells with Parafilm M and incubate at room temperature for 1-2 hours.

4. Aspirate and wash the wells with 150 µl of **diluted F1** three times.

5. Dilute **F3** (at a 1:1000 ratio) to 1 µg/ml with **F2**. Add 50 µl of diluted **F3** to each well and incubate at room temperature for 60 minutes on an orbital shaker (100 rpm).

6. Aspirate and wash the wells with 150 µl of **diluted F1** six times.

7. Prepare the **Fluoro-Development Solution** by adding 1 µl of **F4** and 1 µl of **F5** into each 400 µl of **F6**. Add 50 µl of **Fluoro-Development Solution** into the wells and incubate at room temperature for 1-5 minutes away from light. The color in the standard wells containing the higher concentrations may turn slightly pink during this period. Measure and read fluorescence on a fluorescence microplate reader at $530_{EX}/590_{EM}$ nm.

   Note: *If the strip well frame does not fit the fluorescence reader, transfer the solution to a standard 96-well microplate and read fluorescence at $530_{EX}/590_{EM}$ nm.*

8. Calculate % histone H3-K4 di-methylation:

$$\text{Di-Methylation \%} = \frac{\text{RFU (treated (tested) sample – blank)}}{\text{RFU (untreated (control) sample – blank)}} \times 100\%$$

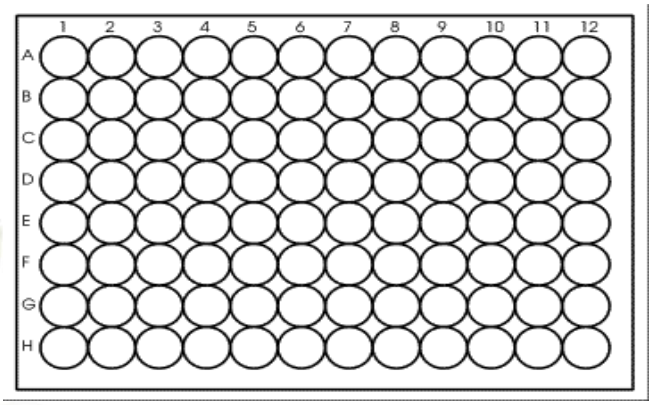
   For the amount quantification, plot RFU versus amount of **Standard Control** and determine the slope as delta RFU/ng.

   Calculate the amount of di-methyl H3-K4 using the following formula:

$$\text{Amount (ng/mg protein)} = \frac{\text{RFU (sample – blank)}}{\text{Protein (µg)*} \times \text{slope}} \times 1000$$

   * *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 (**green trimmed**); 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 duplicates or triplicates.

Appendix

Histone Extraction Protocol

1. *For tissues (treated and untreated),* weigh the sample and cut it into small pieces (1-2 mm^3) with a scalpel or scissors. Transfer tissue pieces to a Dounce homogenizer. Add TEB buffer (PBS containing 0.5% Triton X 100, 2 mM PMSF and 0.02% NaN3) at 1 ml per 200 mg of tissue, and disaggregate tissue pieces by 50-60 strokes. 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 cells (treated and untreated), harvest cells and pellet the cells by centrifugation at 1000 rpm for 5 minutes at 4°C. Resuspend cells in TEB buffer at 10^7 cells/ml and lyse cells on ice for 10 minutes with gentle stirring. Centrifuge at 3000 rpm for 5 minutes at 4°C. If total volume is less than 2 ml, transfer cell lysates to a 2 ml vial and centrifuge at 10,000 rpm for 1 minute at 4°C. Remove supernatant.

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

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

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

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

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 proper 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 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 a 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 a sufficient amount of protein.
Protein extracts are incorrectly stored.	Ensure the protein extracts are stored at –20°C or –80°C.

High Background Present for the Blank

The well is not washed sufficiently.	Check if wash at each step is performed according to the protocol.
Contaminated by the standard control.	Ensure the well is not contaminated from adding the control protein or from using control protein contaminated tips.
Overdevelopment.	Decrease development time in Step 7.

RELATED PRODUCTS

P-3002	*EpiQuik*™ Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K4)
P-3015	*EpiQuik*™ *In Situ* Histone H3-K4 Methylation Assay Kit
P-3022	*EpiQuik*™ Global Di-Methyl Histone H3-K4 Quantification Kit (Colorimetric)
P-3024	*EpiQuik*™ Global Mono-Methyl Histone H3-K4 Quantification Kit (Colorimetric)
P-3025	*EpiQuik*™ Global Mono-Methyl Histone H3-K4 Quantification Kit (Fluorometric)
P-3026	*EpiQuik*™ Global Tri-Methyl Histone H3-K4 Quantification Kit (Colorimetric)

P-3027	*EpiQuik*™ Global Tri-Methyl Histone H3-K4 Quantification Kit (Fluorometric)
P-3028	*EpiQuik*™ Global Pan-Methyl Histone H3-K4 Quantification Kit (Colorimetric)
P-3029	*EpiQuik*™ Global Pan-Methyl Histone H3-K4 Quantification Kit (Fluorometric)

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