Version 1.0908

EpiQuik™ Global Pan-Methyl Histone H4-K20 Quantification Kit (Fluorometric)

Catalog No. P-3071

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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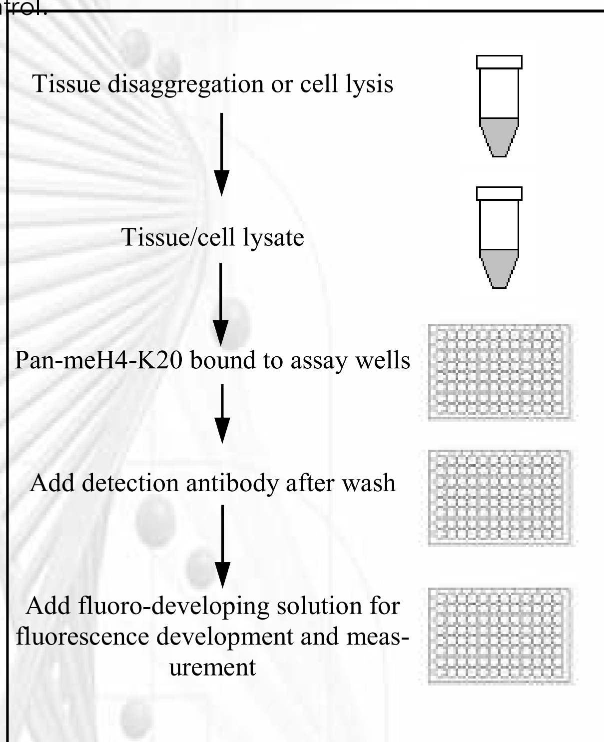
INTRODUCTION

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 transcriptional repression or activation. HMTs transfer 1-3 methyl groups from S-adenosyl-L-methionine to the lysine and arginine residues of histone proteins. PR-SET7, SET9, SUV4.20h, and ASH1 are histone methyltransferases that catalyze methylation of histone H4 at lysine 20 (H4-K20) in mammalian cells. H4-K20 mono-methylation is involved in the maintenance of proper higher order structure of DNA and is consequently essential for chromosome condensation, as well as functioning in gene silencing. H4-K20 di-methylation has been described as a repressive chromatin domain and is involved in DNA damage response. H4-K20 tri-methylation acts as a passive feature or structure determinant for chromatin degradation and release and is an epigenetic marker of early apoptosis. Tri-methylation of H4-K20 is also considered to be a common hallmark of human cancer. The global H4-K20 methylation can be changed by inhibition or activation of HMTs. Thus, quantitative detection of global methyl histone H4-K20 would provide useful information for better understanding epigenetic regulation of gene activation/repression, as well as for developing HMT-targeted drugs. The *EpiQuik*™ Global Pan-Methyl Histone H4-K20 Quantification Kit (Fluorometric) provides a tool for measuring global mono-, di-, and tri-methylation of histone H4-K20. The kit has the following features:

- Quick and efficient procedure, which can be finished within 2.5 hours.
- Innovative fluorometric assay without the need for radioactivity, electrophoresis, or chromatography.
- Simultaneously quantify mono-, di-, and tri-methylated H4-K20 with the detection limit as low as 1 ng/well, and detection range from 10 ng-2 μg/well of histone extracts.
- The control is conveniently included for the quantification of pan-methylated H4-K20.
- Strip microplate format makes the assay flexible: manual or high throughput.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE AND PROCEDURE

The *EpiQuik*™ Global Pan-Methyl Histone H4-K20 Quantification Kit (Fluorometric) is designed for simultaneously measuring mono-, di-, and tri-methylation of histone H4-K20. In an assay with this kit, the mono-, di-, and tri-methylated histone H4 at lysine 20 is captured to the strip wells coated with an anti-mono-, di-, and tri-methyl H4-K20 antibody. The captured methylated histone H4-K20 can then be detected with a detection antibody followed by a fluorescent development reagent. The ratio of mono-, di-, and tri-methylated H4-K20 is proportional to the intensity of fluorescence. The absolute amount of mono-, di-, and tri-methylated H4-K20 can be quantitated by comparing to the standard control.



Schematic Procedure for Using the *EpiQuik*™ Global Pan-Methyl Histone H4-K20 Quantification Kit (Fluorometric)

PRODUCT USE INFORMATION

The *EpiQuik™* Global Pan-Methyl Histone H4-K20 Quantification Kit (Fluorometric) is suitable for specifically measuring global histone H4-K20 tri-methylation using a variety of mammalian cells (human, mouse, etc.) including fresh and frozen tissues, cultured adherent and suspension cells.

The *EpiQuik™* Global Pan-Methyl Histone H4-K20 Quantification Kit (Fluorometric) 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 Pan-Methyl Histone H4-K20 Quantification Kit (Fluorometric) and methods of use are covered by a pending US patent.

KIT CONTENTS

Components	96 assays P-3071-96
F1 (10X wash buffer)	20 ml
F2 (antibody buffer)	12 ml
F3 (detecting antibody, 1 mg/ml)*	10 µl
F4 (fluoro developer)*	24 µl
F5 (fluoro enhancer)*	24 µl
F6 (fluoro dilution)	8 ml
Standard control (100 µg/ml)*	20 µl
Signal report solution*	10 µl
Signal enhancer*	240 µl
8 well sample strips (with frame)	9
8 well standard control strips	3
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** For maximum recovery of the products, centrifuge the original vial prior to opening the cap.*

SHIPPING AND STORAGE

Upon receipt, store **F4** and **standard control** at –20°C. Store **all other components** at 4°C away from light. The components of the kit are stable for 6 months 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

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 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 1:9 ratio (1 ml of **F1** + 9 ml of distilled water).

3. Add 50 µl of **F2** 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 **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 **standard control** at the different concentrations into the standard well. 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 hour.

   Meanwhile, prepare **detection solution**: for each 1 ml of **detection solution** to be prepared, add 1 µl of **F3** and 0.5 µl of **signal report solution** into 10 µl of **diluted F1**, mix and incubate at room temperature for 10 min. Then add 20 µl of signal enhancer, mix and incubate at room temperature for 15 min. Finally add 970 µl of **diluted F1** and mix.

4. Aspirate and wash the wells with 150 µl of **diluted F1** 3 times.

5. Add 50 µl of the **detection solution** 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 F1** 6 times.

7. Prepare **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 min away from light. The color in the standard wells containing the higher concentrations may turn slightly pink during this period. Measure and read fluorescence with a fluorescence microplate reader at $530_{EX}/590_{EM}$ nm.

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

8. Calculate % histone H4-K20 mono-, di-, or tri-methylation:

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

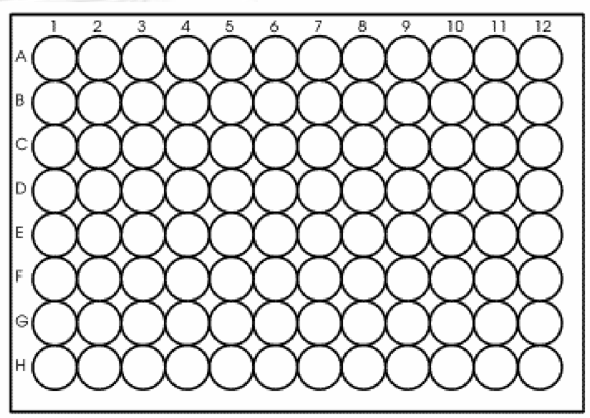
For the amount quantification, plot delta RFU versus amount of **standard control** and determine the slope as delta RFU/ng.

Calculate the amount of mono-, di-, and tri-methylated H4-K20 using the following formula:

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

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

Plate Configuration



- **Strip 1-3** : standard wells (**Labeled as SC**); 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 :** Sample well**: 4-6: mono-methyl; 7-9: di-methyl; 10-12: tri-methyl**.
- 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 the sample 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 200 mg/ml, and disaggregate tissue pieces by 50-60 strokes. Transfer homogenized mixture to a 15 ml conical tube and centrifuge at 3000 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 10,000 rpm for 1 min at 4°C. Remove supernatant.

2. Resuspend cell/tissue pellet in 3 volumes (approx. 200 μl/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 a 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 μl/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

Problem	Solution
Reagents are added incorrectly.	Check if reagents are added in the proper 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 followed correctly.

No Signal or Very Weak Signal for Only the Standard Control

Problem	Solution
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

Problem	Solution
The protein sample is not extracted properly.	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 protein.
Protein extracts are incorrectly stored.	Ensure the protein extracts are stored at –20°C or –80°C.

High Background Present for the Blank

Problem	Solution
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 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 Pan-Methyl Histone H4-K20 Quantification Kit (Fluorometric)	48 assays 96 assays	P-3071-48 P-3071-96

Available Related Products	Cat. No.
EpiQuik™ Global Mono-Methyl Histone H4-K20 Quantification Kit (Colorimetric)	P-3064
EpiQuik™ Global Mono-Methyl Histone H4-K20 Quantification Kit (Fluorometric)	P-3065
EpiQuik™ Global Di-Methyl Histone H4-K20 Quantification Kit (Colorimetric)	P-3066
EpiQuik™ Global Di-Methyl Histone H4-K20 Quantification Kit (Fluorometric)	P-3067
EpiQuik™ Global Tri-Methyl Histone H4-K20 Quantification Kit (Colorimetric)	P-3068
EpiQuik™ Global Tri-Methyl Histone H4-K20 Quantification Kit (Fluorometric)	P-3069
EpiQuik™ Global Pan-Methyl Histone H4-K20 Quantification Kit (Colorimetric)	P-3070

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

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