EPIGENTEK

Version 2.0902

EpiQuik™ Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K4)

Catalog No. P-3002

User Guide*

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

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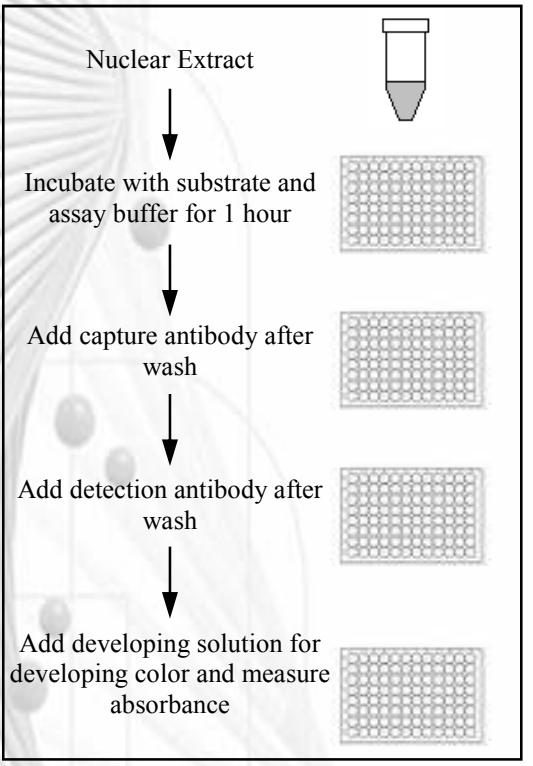
INTRODUCTION

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. Inhibition of HMTs may lead to expression of the silenced genes, and HMT inhibitors are currently developed for various therapeutic or experimental applications. 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 methylation may serve as a global epigenetic mark in euchromatin and mediates activated transcription. There is only the radioisotopic method currently available for measuring HMT activity/ inhibition, which is time consuming, labor-intensive, and has low throughput or produces radioactive waste. The *EpiQuik*™ Histone Methyltransferase Activity/ Inhibition Assay Kit (H3-K4) addresses these problems by using a unique procedure to measure HMT activity/inhibition. 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.
- Specific measurement of activity/inhibition of H3-K4 histone methyltransferases.
- Strip microplate format makes the assay flexible: manual or high throughput analysis.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE AND PROCEDURE

The *EpiQuik*™ Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K4) is designed for measuring HMTs that specifically target histone H3 at lysine 4. In an assay with this kit, the histone substrate is stably captured on the strip wells. HMT enzymes transfer a methyl group to histone H3 substrate from Adomet to methylate the substrate at lysine 4. The methylated histone H3-K4 can then be recognized with a high-affinity antibody. The ratio or amount of methylated H3-K4, which is directly proportional to enzyme activity, can be quantified through HRP conjugated secondary antibody-color development system. The HMT activity is then calculated based on the amount of methylated H3-K4 converted by the HMTs.



Schematic Procedure for Using the *EpiQuik*™ Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K4)

PRODUCT USE INFORMATION

The *EpiQuik*™ Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K4) is very suitable for specifically measuring activity/inhibition of individual histone methyltransferase targeting lysine residues at different sites.

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.

The *EpiQuik*™ Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K4) is for research use only and is not intended for diagnostic or therapeutic application.

EpiQuik™ is a trademark of Epigentek Group Inc.

The *EpiQuik*™ Histone Methyltransferase Activity/Inhibition Assay Kits and methods of use are covered by a pending US patent.

KIT CONTENTS

Components	48 assays P-3002-1	96 assays P-3002-2
HM1 (10X Wash Buffer)	11 ml	22 ml
HM2 (Histone Assay Buffer)	1.5 ml	3 ml
HM3 (Adomet)*	25 µl	50 µl
HM4 (Biotinylated Substrate, 25 µg/ml)*	100 µl	200 µl
HM5 (HMT Standard, 10 µg/ml)*	10 µl	20 µl
HM6 (Capture Antibody, 100 µg/ml)*	25 µl	50 µl
HM7 (Detection Antibody, 200 µg/ml)*	10µl	20 µl
HM8 (Developing Solution)	6 ml	12 ml
HM9 (Stop Solution)	3 ml	6 ml
Control Enzyme (100 µg/ml)*	10 µl	20 µl
8-Well Assay Strip (with frame)	6	12
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** For maximum recovery of the products, centrifuge the original vial after thawing 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 **HM3**, **HM4**, **HM5**, **HM7**, and the **Control Enzyme** at –20°C away from light; (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 wash buffer, **HM1**, contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are redissolved.

MATERIALS REQUIRED BUT NOT SUPPLIED

Orbital shaker
Pipettes and pipette tips
Microplate reader
1.5 ml microcentrifuge tubes
Distilled water

PROTOCOL

1. Prepare nuclear extracts by using your own successful method. For your convenience and the best results, Epigentek offers a nuclear extraction kit (Cat. No. OP-0002-1) optimized for use with the *EpiQuik™* series. Nuclear 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 **HM1** 10X Wash Buffer with distilled water (pH 7.2-7.5) at a 1:10 ratio (e.g., 1 ml of **HM1** + 9 ml of distilled water).
3. Dilute **HM3** with **HM2** (at a 1:5 ratio). Add 24 µl of **HM2**, 1.5 µl of the **diluted HM3**, and 2 µl of **HM4** to each strip well. Then add 3 µl of nuclear extracts (4-20 µg) or HMT enzymes, mix and cover the strip wells with Parafilm M and incubate at 37°C for 60-90 minutes.
   For HMT inhibition, add 3 µl of tested inhibitors at different concentrations and reduce **HM2** volume to 21 µl. For the blank, add 3 µl of **HM2** instead of nuclear extracts. For the standard curve, add 3 µl of **HM2** instead of nuclear extracts, and add 2 µl of **HM5** at different concentrations (ex: 0.1 – 5 ng/µl) instead of **HM4**. A positive control can be optionally set up by adding 1-2 µl of the **Control Enzyme** and 2 µl of **HM2** instead of nuclear extracts.
4. Aspirate and wash each well with 150 µl of **diluted HM1** three times.
5. Dilute the **HM6** (at a 1:200 ratio) to 0.5 µg/ml with **diluted HM1**. Add 50 µl of **diluted HM6** to each strip well and incubate at room temperature for 60 minutes on an orbital shaker (50-100 rpm).
6. Aspirate and wash each well with 150 µl of **diluted HM1** five times.
7. Dilute **HM7** (at a 1:1000 ratio) with **diluted HM1**. Add 50 µl of the **diluted HM7** to each strip well and incubate at room temperature for 30 minutes.
8. Aspirate and wash each well with 150 µl of **diluted HM1** five times.
9. Add 100 µl of **HM8** into 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 **HM9** to each well to stop enzyme reaction when the 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 minutes.

(Continued on Next Page)

11. Calculate HMT activity or inhibition. For simple calculation use the following formula:

$$\text{Activity (O.D./h/mg)} = \frac{\text{OD (no inhibitor – blank)}}{\text{Protein amount } (\mu g)^{*} \times \text{hour}^{**}} \times 1000$$

$$\text{Inhibition \%} = \left(1 - \frac{\text{OD (inhibitor sample – blank)}}{\text{OD (no inhibitor control – blank)}}\right) \times 100\%$$

For accurate calculation, plot OD value versus amount of **HM5** and determine the slope as delta OD/ng and calculate HMT activity using the following formula:

$$\text{Activity (ng/h/mg)} = \frac{\text{OD (sample – blank)}}{\text{Protein Amount } (\mu g) \times \text{hour} \times \text{slope}} \times 1000$$

* Protein amount added into the reaction at step 3.
** Incubation time at step 3.

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 in the protocol are followed correctly.

No Signal or Very Weak Signal for Only the Positive Control

The positive control enzyme is insufficiently added to the well.	Ensure a sufficient amount of control enzyme is added.
The positive control enzyme have lost activity due to incorrect storage.	Follow the guidance in the protocol for storage of the positive control.

No Signal for Only the Sample

The protein sample is not properly extracted.	Ensure the nuclear protein extraction protocol is suitable for HMT protein extraction. Sodium chloride concentration of the extraction buffer should not be more than 100 mM.
The protein amount is added into well insufficiently.	Ensure extract contains a sufficient amount of protein.
The sample is not prepared from fresh cells or tissues.	The nuclear extracts from frozen cells or tissues significantly lose enzyme activity. Fresh samples should be used.
Nuclear extracts are incorrectly stored or have been stored for a long duration.	Ensure the nuclear extracts are stored at –80°C for no more than 6 weeks.

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 positive control or HMT standard.	Ensure the well is not contaminated from adding the control enzyme or HMT standard accidentally, or by using enzyme or HMT standard contaminated tips.
Overdevelopment.	Decrease development time in protocol step 9.

ORDERING INFORMATION

Products	Size	Cat. No.
EpiQuik™ Histone Methyltransferase Activity/ Inhibition Assay Kit (H3-K4)	48 assays 96 assays	P-3002-1 P-3002-2

Available Related Products	Cat. No.
EpiQuik™ DNA Methyltransferase Activity/Inhibition Assay Kit	P-3001
EpiQuik™ Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K9)	P-3003
EpiQuik™ Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K27)	P-3005

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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