GENTAUR

DATA SHEET

EpiQuik Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K27)

Base Catalog # P-3005

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik*™ Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K27) is very suitable for specifically measuring the activity/inhibition of individual histone methyltransferase targeting to lysine residues at different sites.

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

KIT CONTENTS

Components	48 assays P-3005-48	96 assays P-3005-96
HE1 (10X Wash Buffer)	11 ml	22 ml
HE2 (Histone Assay Buffer)	1.5 ml	3 ml
HE3 (Adomet)*	25 µl	50 µl
HE4 (Biotinylated Substrate, 25 µg/ml)*	100 µl	200 µl
HE5 (HMT Standard, 10 µg/ml)*	10 µl	20 µl
HE6 (Capture Antibody, 100 µg/ml)*	25 µl	50 µl
HE7 (Detection Antibody, 100 µg/ml)*	10 µl	20 µl
HE8 (Developing Solution)	6 ml	12 ml
HE9 (Stop Solution)	3 ml	6 ml
Control Enzyme (300 µg/ml)*	5 µl	10 µl
8-Well Assay Strips (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 & 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 HE3, HE4, HE5, HE7, 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, HE1, 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
- ☐ Orbital shaker
- ☐ Distilled water

GENERAL PRODUCT INFORMATION

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.

Usage Limitation: The *EpiQuik*™ Histone Methyltransferase Activity/Inhibition Assay Kits are for research use only and are not intended for diagnostic or therapeutic application.

Intellectual Property: The *EpiQuik*™ Histone Methyltransferase Activity/Inhibition Assay Kits and method of use contain proprietary technologies by Epigentek. *EpiQuik*™ is a trademark of Epigentek Group Inc.

A BRIEF OVERVIEW

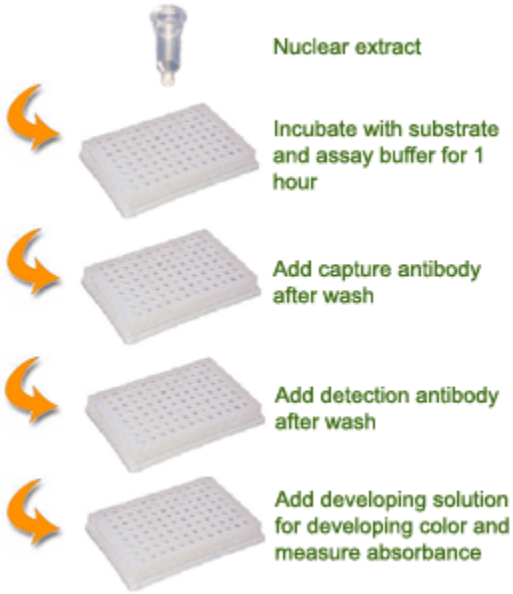
Epigenetic inactivation of gene play a critical role in many important human diseases, especially in cancer. A major mechanism for epigenetic inactivation of the genes is methylation of CpG islands in genome DNA caused by DNA methyltransferases. Histone methyltransferases (HMTs) control or regulate DNA 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. Inhibition of HMTs may lead to expression of the silenced genes and HMT inhibitors are currently developed for various therapeutic or experimental applications. 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. H3-K27 methylation mediates heterochromatin formation by forming a binding site for polycomb, and also participates in silencing gene expression at euchromatic sites. Increased global H3-K27 methylation is also found to be involved in some pathological processes such as cancer progression. There is no method currently used for measuring HMT activity/inhibition (H3-K27). The *EpiQuik*™ Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K27) addresses this problem by using a unique procedure to measure HMT activity/inhibition (H3-K27). 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-K27 histone methyltransferases.
- Strip microplate format makes the assay flexible: manual or high throughput analysis.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The *EpiQuik*™ Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K27) is designed for measuring HMTs that specifically target histone H3 at lysine 27. In an assay with this kit, the histone substrate is stably captured on the strip wells through biotin-streptavidin binding. HMT

enzymes transfer a methyl group to histone H3 substrate from Adomet to methylate the substrate at lysine 27. The methylated histone H3-K27 can be recognized with a high-affinity antibody. The ratio or amount of methylated H3-K27, 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-K27 converted by the HMTs.



Schematic Procedure for Using the *EpiQuik*™ Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K27)

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 **HE1** with distilled water (pH 7.2 to 7.5) at a 1:10 ratio (ex: 1 ml of **HE1** + 9 ml of distilled water).
3. Dilute **HE3** with **HE2** (at a 1:5 ratio). Add 24 µl of **HE2**, 1.5 µl of the diluted **HE3**, and 2 µl of **HE4** 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 minutes.
   For HMT inhibition, add 3 µl of tested inhibitors at different concentrations and reduce **HE2** volume to 21 µl. For the blank, add 3 µl of **HE2** instead of nuclear extracts. For the standard curve, add 3 µl of **HE2** instead of nuclear extracts and add 2 µl of **HE5** at different concentrations (ex: 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0 ng/µl) instead of **HE4**. A positive control can be optionally set up by adding 0.5-1 µl of **Control Enzyme** instead of nuclear extracts.
4. Aspirate and wash each well with 150 µl of **diluted HE1** three times.
5. Dilute the **HE6** (at a 1:100-1:200 ratio) with **diluted HE1**. Add 50 µl of **diluted HE6** 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 HE1** four times.

7. Dilute the **HE7** (at a 1:1000 ratio) with **diluted HE1**. Add 50 µl of **diluted HE7** to each strip well and incubate at room temperature for 30 minutes.
8. Aspirate and wash each well with 150 µl of **diluted HE1** four times. Allow 3 minutes for last wash.
9. Add 100 µl of **HE8** 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 **HE9** 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.
11. Calculate HMT activity or inhibition. For simple calculation:

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

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

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

For accurate calculation, plot OD value versus the amount of **HE5** and determine the slope as delta OD/ng.

Calculate HMT activity using the following formula:

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

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 enzyme is insufficiently added to the well.	Ensure a sufficient amount of control enzyme is added.
The positive control enzyme has 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 loses enzyme activity. A fresh sample should be used.
Nuclear extracts are incorrectly stored or have been stored for a long period of time.	Ensure the nuclear extracts are stored at –80°C for no more than 6 weeks.
Absence of HMT activity in the sample due to treatment.	N/A.

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 from using enzyme or HMT standard contaminated tips.
Overdevelopment.	Decrease development time in step 9 of "target protein level detection."

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