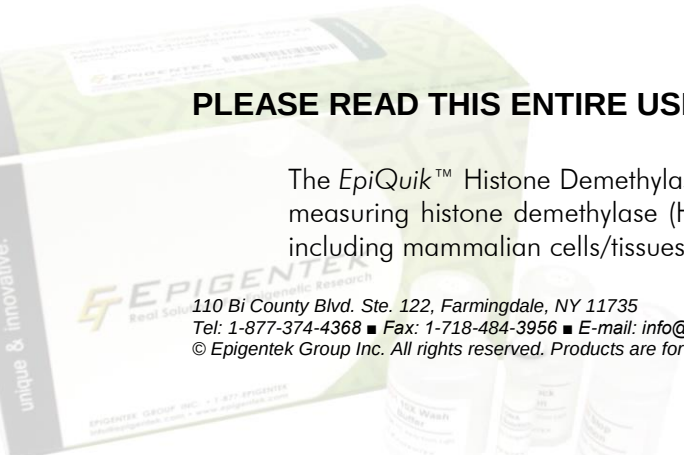
# EpiQuik™ Histone Demethylase (H3-K4 Specific) Activity/Inhibition Assay Kit

Base Catalog # P-3074



**PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE**

The *EpiQuik*™ Histone Demethylase (H3-K4 Specific) Activity/Inhibition Assay Kit is very suitable for measuring histone demethylase (H3-K4 specific) activity/inhibition from a broad range of species including mammalian cells/tissues, plants, and bacteria.

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## KIT CONTENTS

| Components | 48 assays P-3074-48 | 96 assays P-3074-96 |
|---|---|---|
| HD1 (10X Wash Buffer) | 15 ml | 30 ml |
| HD2 (HDM Assay Buffer) | 1.5 ml | 3 ml |
| HD3 (HDM Substrate)* | 50 µl | 100 µl |
| HD4 (HDM Standard, 20 µg/ml)* | 25 µl | 50 µl |
| HD5 (Capture Antibody, 1000 µg/ml)* | 5 µl | 10 µl |
| HD6 (Detection Antibody, 200 µg/ml)* | 8 µl | 16 µl |
| HD7 (Fluoro Developer)* | 12 µl | 24 µl |
| HD8 (Fluoro Enhancer)* | 12 µl | 24 µl |
| HD9 (Fluoro Dilution) | 4 ml | 8 ml |
| 8-Well Assay Strip (with Frame) | 6 | 12 |
| User Guide | 1 | 1 |

** 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 HD3, HD4, HD6, and HD7 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, HD1, 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
- ☐ Fluorescent microplate reader
- ☐ 1.5 ml microcentrifuge tubes

## GENERAL PRODUCT INFORMATION

**Usage Limitation:** The *EpiQuik*™ Histone Demethylase (H3-K4 Specific) Activity/Inhibition Assay Kit 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*™ Histone Demethylase (H3-K4 Specific) Activity/Inhibition Assay Kit and methods of use contain proprietary technologies by Epigentek. *EpiQuik*™ is a trademark of Epigentek Group Inc.

## A BRIEF OVERVIEW

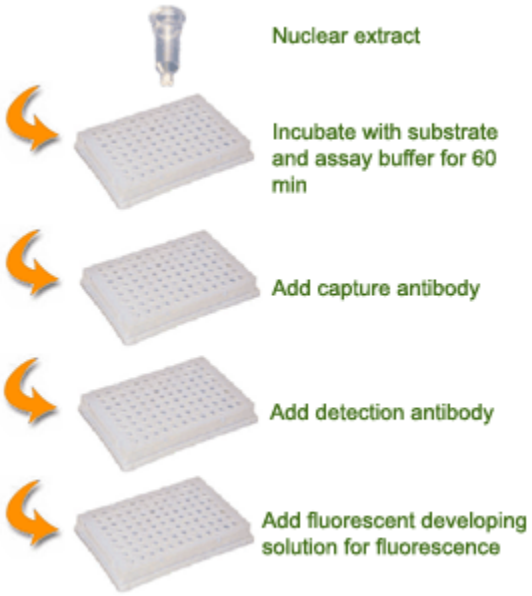
Lysine histone methylation is one of the most robust epigenetic marks and is essential for the regulation of multiple cellular processes. The methylation of H3-K4 seems to be of particular significance, as it is associated with active regions of the genome. H3-K4 methylation was considered irreversible until the identification of a large number of histone demethylases indicated that demethylation events play an important role in histone modification dynamics. So far, at least 2 classes of H3-K4 specific histone demethylase, LSD1 and JARIDs, have been identified. LSD1 can remove di- and mono-methylation from H3-K4 by using an amine oxidase reaction, while JARIDs such as RBP2, PLU-1, SMCX, and SMCY catalyzes the removal of methylation by using a hydroxylation reaction and required iron and α-ketoglutarate as cofactors. H3-K4 specific demethylases are found to be involved in some pathological processes such as cancer progression. Inhibition of the enzymes may lead to re-methylation of H3-K4 and silencing of H3-K4 enriched active genes. There are few methods currently available for measuring activity/inhibition of H3-K4 specific methylases using a variety of cells/tissues.

The *EpiQuik*™ Histone Demethylase (H3-K4 Specific) Activity/Inhibition Assay Kit uses a proprietary and unique procedure to measure activity/inhibition of H3-K4 specific histone demethylases using cell/tissue extracts. This kit has the following features:

- Fast procedure, which can be finished within 3 hours.
- Innovative fluorescent assay without the need for radioactivity, extraction, or chromatography.
- Direct measurement of HDM (H3-K4 specific) activity and inhibition without interference by thiol-containing chemicals such as DTT, GSH, and 2-mercaptoethanol.
- Strip microplate format makes the assay flexible: manual or high throughput analysis.
- Simple, reliable, and consistent assay conditions.

## PRINCIPLE & PROCEDURE

The *EpiQuik*™ Histone Demethylase (H3-K4 Specific) Activity/Inhibition Assay Kit is designed for measuring total histone demethylase (H3-K4 specific) activity/inhibition. In the assay with this kit, the unique di-methylated histone H3-K4 substrate is stably captured on the strip wells. Active HDMs bind to and demethylate histone H3-K4 substrate. The remaining un-demethylated substrate can be recognized with a high affinity anti-methylated histone H3-K4 antibody. The ratio or amount of the un-demethylated histone, which is inversely proportional to HDM enzyme activity, can then be fluorometrically quantified.




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

## PROTOCOL

1. Prepare nuclear extracts by using you own successful method. For your convenience and the best results, Epigentek offers a nuclear extraction kit (Cat. No. OP-0002-1) optimized for use in *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 **HD1** 10X Wash Buffer with distilled water (pH 7.2 to 7.5) into a 1X concentration (**1X HD1**).

3. Dilute **HD3** at a 1:50 ratio with **1X HD1**, and add 50 µl of the **diluted HD3** into each well. For preparation of the standard curve, add 50 µl of **1X HD1** into the wells (no **HD3** added), followed by adding 1 µl of **HD4** at different concentrations (0.1 – 10 ng). Cover the wells with Parafilm M and incubate at room temperature for 30-45 minutes.

4. Aspirate and wash each well with 150 µl of **1X HD1** two times.

5. Add 28 µl of **HD2** and 2 µl of nuclear extracts (4-20 µg) to each strip well. Mix, cover the strip wells, and incubate at 37°C for 60 minutes. For the control, add 2 µl of **HD2** instead of nuclear extract. For HDM inhibition, add 2 µl of tested inhibitors at different amounts and reduce **HD2** volume to 26 µl. For the blank, add 30 µl of **HD2** into the blank wells (no **HD3** added).

6. Aspirate and wash each well with 150 µl of **1X HD1** three times.

7. Dilute **HD5** (at a 1:1000 ratio) to 1 µg/ml with **1X HD1**. Add 50 µl of the **diluted HD5** to each strip well and incubate at room temperature for 60 minutes on an orbital shaker (50-100 rpm).

8. Aspirate and wash each well with 150 µl of **1X HD1** four times.

9. Dilute **HD6** (at a 1:1000 ratio) to 0.2 µg/ml with **1X HD1**. Add 50 µl of the **diluted HD6** to each strip well and incubate at room temperature for 25-30 min.

10. Aspirate and wash each well with 150 µl of **1X HD1** five to six times.

11. Prepare the **Fluoro-Development Solution** by adding 1 µl of **HD7** and 1 µl of **HD8** into each 400 µl of **HD9**.

12. Add 50 µl of the **Fluoro-Development Solution** into the wells and incubate at room temperature for 2-5 minutes away from light. 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.*

13. Calculate HDM (H3-K4) activity or inhibition. For simple calculation:

$$\text{HDM activity (RFU/h/}\mu\text{g)} = \frac{\text{[RFU (control – blank) – RFU (sample – blank)]}}{\text{reaction time (0.5-1 h) x protein amount added (}\mu\text{g)}}$$

$$\text{Inhibition \%} = \left(1 - \frac{\text{[RFU (control – blank) – RFU (inhibitor sample – blank)]}}{\text{[RFU (control – blank) – RFU (no inhibitor sample – blank)]}}\right) \text{x } 100\%$$

For an accurate calculation, plot Delta RFU value versus amount of **HD4** and determine the slope as delta RFU/ng.

Calculate HDM (H3-K4) activity using the following formula:

$$\text{Activity (ng/h/}\mu\text{g)} = \frac{\text{[RFU (control-blank) – RFU (sample – blank)]}}{\text{slope x reaction time (1 h) x protein amount added (}\mu\text{g)}}$$

## TROUBLESHOOTING

### No Signal for the Sample

| | |
|---|---|
| The protein sample is not properly extracted. | Ensure the protein extraction protocol is suitable for nuclear protein extraction. |
| The protein amount is added into | Ensure extract contains a sufficient amount of |

| | |
|---|---|
| well insufficiently. | protein. |
| The sample is not prepared from frozen cells or tissues. | The nuclear extracts from frozen cells/tissue significantly lose enzyme activity. A fresh sample should be used. |
| Nuclear extracts are incorrectly stored. | Ensure the nuclear extracts are stored at –80°C. |
| 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 are incorrect. | Ensure the incubation time and temperature described in the protocol are followed correctly. |
| Absence of HDM (H3-K4) 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. |
| Overdevelopment. | Decrease development time in step 12. |

## RELATED PRODUCTS

| | |
|---|---|
| P-3075A | EpiQuik Histone Demethylase LSD1 Inhibitor Screening Assay Core Kit |
| P-3076 | *EpiQuik*™ Histone Demethylase LSD1 Activity/Inhibition Assay Kit |