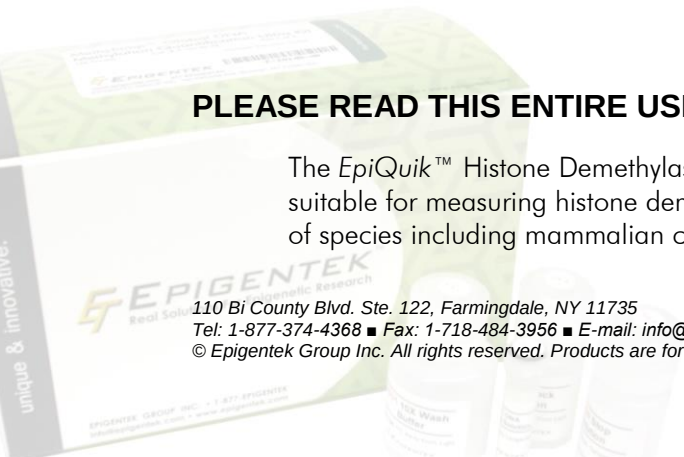
EpiQuik™ Histone Demethylase (H3-K9 Specific) Activity/Inhibition Fast Assay Kit

Base Catalog # P-3077



PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik*™ Histone Demethylase (H3-K9 Specific) Activity/Inhibition Fast Assay Kit is very suitable for measuring histone demethylase (H3-K9 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-3077-48	96 assays P-3077-96
HL1 (HDM Assay Buffer)	2 ml	4 ml
HL2 (HDM Substrate)*	50 µl	100 µl
HL3 (HDM Standard, 10 mM)*	25 µl	50 µl
HL4 (Fluoro-Developer)	4 ml	8 ml
HL5 (Fluoro-Enhancer)	4 ml	8 ml
HDM Cofactor 1*	25 µl	50 µl
HDM Cofactor 2*	25 µl	50 µl
HDM Cofactor 3*	25 µl	50 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 & 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 HL2 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 date of shipment, when stored properly.

Note: *Tightly cap HL4 (Fluoro-Developer) after each use*

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-K9 Specific) Activity/Inhibition Fast 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-K9 Specific) Activity/Inhibition Fast Assay Kit and methods of use contain proprietary technologies by Epigentek. *EpiQuik*™ is a trademark of Epigentek Group Inc.

A BRIEF OVERVIEW

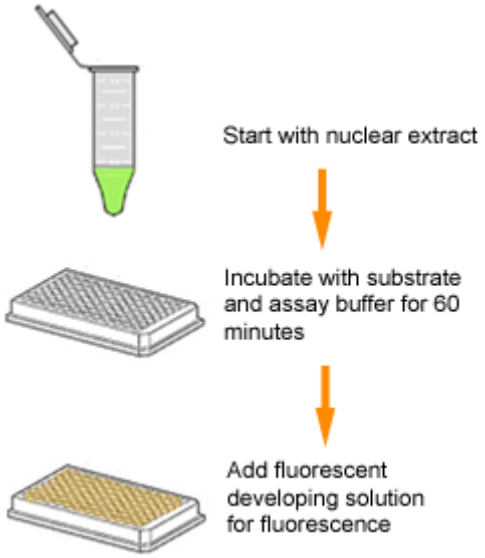
Lysine histone methylation is one of the most robust epigenetic marks, and is essential for the regulation of multiple cellular processes. The methylation of H3-K9 seems to be of particular significance as it is associated with repression regions of the genome. H3-K9 methylation was considered irreversible until 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-K9 specific histone demethylase– JMJD1(JHDM2) and JMJD2 (JHDM3)– have been identified. The JMJD1 family (including JMJD1A, JMJD1B, and JMJD1C) can remove di- and mono-methylation from H3-K9; while the JMJD2 family (including JMJD2A, JMJD2B, JMJD2C, and JMJD2D) can remove tri-methylation from H3-K9. Both, JMJD1 and JMJD2, catalyze the removal of methylation by using a hydroxylation reaction with a required iron and α-ketoglutarate as cofactors. Lastly, H3-K9 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-K9 and activation of H3-K9 enriched repression genes. Currently, there are few methods available for measuring activity/inhibition of H3-K9 specific methylases using a variety of cells/tissues.

The *EpiQuik*™ Histone Demethylase (H3-K9 Specific) Activity/Inhibition Fast Assay Kit uses a proprietary and unique procedure to measure activity/inhibition of H3-K9 specific histone demethylases using cell/tissue extracts. This kit has the following features:

- Fast one-step assay, which can be finished within 1.5 hours.
- Innovative fluorescent assay without the need for radioactivity, extraction, or chromatography.
- Measurement of HDM (H3-K9 specific) activity and inhibition through quantifying by-product of the enzyme reaction, which makes the assay highly sensitive.
- 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-K9 Specific) Activity/Inhibition Fast Assay Kit is designed for measuring total histone demethylase (H3-K9 specific) activity/inhibition. In the assay with this kit, the unique methylated histone H3-K9 substrate is incubated with nuclear extracts or purified enzymes. Formaldehyde, generated from the enzyme-substrate reaction, reacts with a detection reagent to form a fluorescent substance. The ratio or amount of the formaldehyde, which is proportional to HDM enzyme activity, can then be fluorometrically quantified.



Schematic Procedure for Using the *EpiQuik*™ Histone Demethylase (H3-K9 Specific) Activity/Inhibition Fast Assay Kit

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 in 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).

3. Prepare the **Completed HL1 (CHL1)** by adding **HDM Cofactor 1**, **2**, and **3**, respectively into **HL1** at a 1:100 ratio (ex: add 1 µl of each Cofactor into 100 µl of **HL1**). For the untreated control, add 27 µl of **CHL1**, 2 µl of nuclear extract (5-10 µg) or purified enzyme, and 1 µl of **HL2** to the strip wells. Mix, cover the strip wells with Parafilm M, and incubate at 37°C for 60 minutes. For the blank, add 2 µl of **CHL1** instead of nuclear extract. For HDM inhibition, add 3 µl of the tested inhibitors at different amounts, and reduce **CHL1** volume to 24 µl. For generating the standard curve, dilute **HL3** with **CHL1** at different concentrations (ex: 12.5, 25, 50, 100, and 200 µM). Add 26 µl of **CHL1**, 1 µl of **HL2**, followed by adding 3 µl of the **diluted HL3** into the wells.

Control wells:	CHL1	27 µl
	HL2	1 µl
	Nuclear Extract	2 µl
Inhibitor wells:	CHL1	24 µl
	HL2	1 µl
	Nuclear Extract	2 µl

	Inhibitor	3 µl
Standard wells:	CHL1	26 µl
	HL2	1 µl
	HDM Standard	3 µl
Blank wells:	CHL1	29 µl
	HL2	1 µl

Note: *The inhibitor compound solution and nuclear extract should not have thiol-containing chemicals, such as DTT, GSH, and 2-mercaptoethanol, as the thiol-containing chemicals may interfere with the fluorometric determination.*

4. Prepare the **Fluoro-Development Solution** by mixing **HL4** and **HL5** at a ratio 1:1 (ex: 1 ml of **HL4** + 1 ml of **HL5**).

5. Add 140 µl of the **Fluoro-Development Solution** into the wells and incubate at room temperature for 15-20 minutes away from light. Measure and read fluorescence on a fluorescence microplate reader at $370_{EX}/470_{EM}$ 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 $370_{EX}/470_{EM}$ nm.*

6. Calculate HDM (H3-K9) activity or inhibition. For simple calculation:

$$\text{HDM activity (RFU/h/}\mu\text{g)} = \frac{\text{RFU (untreated control sample – blank)}}{\text{Reaction time (1 h)} \times \text{protein amount added}}$$

$$\text{Inhibition \%} = \left(1 - \frac{\text{RFU (inhibitor sample – blank)}}{\text{RFU (untreated control sample – blank)}}\right) \times 100\%$$

For an accurate calculation, plot Delta RFU (sample – blank) value versus amount of **HL3** and determine the slope as delta RFU/ng.

Calculate HDM (H3-K9) activity using the following formula:

$$\text{Activity (}\mu\text{M/h/}\mu\text{g)} = \frac{\text{RFU (untreated control – blank)}}{\text{Slope x reaction time (1 h) x protein amount added (}\mu\text{g)}}$$

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 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/tissue significantly lose enzyme activity. Fresh samples should be used.
Nuclear extracts are stored incorrectly.	Ensure the nuclear extracts are stored at –80°C.
Reagents are added incorrectly.	Check if reagents are added in the proper 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.
Absence of HDM (H3-K9) activity in the sample due to treatment.	N/A.

High Background Present for the Blank

Contaminated by nuclear extracts or HDM standard.	Ensure the well is not contaminated from adding the nuclear extract or HDM standard accidentally or from using HDM standard contaminated tips.
Over-development.	Decrease development time in step 5.

RELATED PRODUCTS

P-3074	*EpiQuik*™ Histone Demethylase (H3-K4 Specific) Activity/Inhibition Assay Kit
P-3075A	*EpiQuik*™ Histone Demethylase LSD1 Inhibitor Screening Assay Core Kit
P-3076	*EpiQuik*™ Histone Demethylase LSD1 Activity/Inhibition Assay Kit