EpiQuik™ Histone Demethylase LSD1 Activity/Inhibition Assay Kit

Base Catalog # P-3076

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik*™ Histone Demethylase LSD1 Activity/Inhibition Assay Kit is very suitable for measuring histone demethylase LSD1 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-3076-48	96 assays P-3076-96
HG1 (10X Wash Buffer)	15 ml	30 ml
HG2 (LSD1 Assay Buffer)	2 ml	4 ml
HG3 (LSD1 Substrate)*	50 µl	100 µl
HG4 (LSD1 Assay Standard, 20 µg/ml)*	25 µl	50 µl
HG5 (Capture Antibody, 1000 µg/ml)*	5 µl	10 µl
HG6 (Detection Antibody, 200 µg/ml)*	8 µl	16 µl
HG7 (Fluoro-Developer)	6 ml	12 ml
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 HG3, HG4, and HG6, at –20°C away from light; (2) Store all other components (HG1, HG2, HG5, HG7, and 8-Well Assay Strip) at 4°C away from light. The kit is stable for up to 6 months from the shipment date, when stored properly.

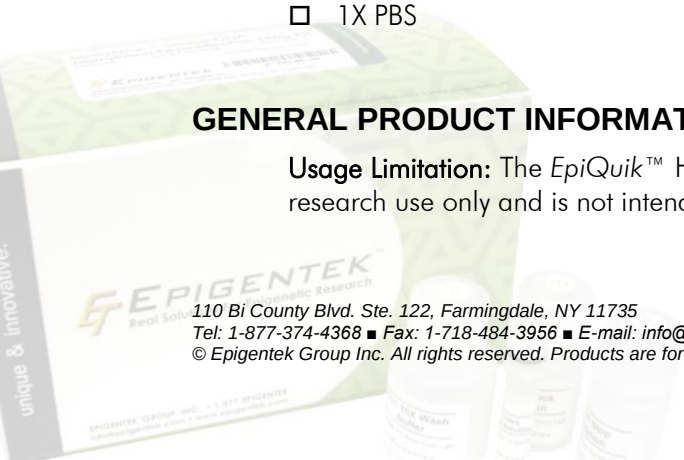
Note: Check if wash buffer, HG1, contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Orbital shaker
- ☐ Pipettes and pipette tips
- ☐ Fluorescent microplate reader
- ☐ 1.5 ml microcentrifuge tubes
- ☐ 1X PBS

GENERAL PRODUCT INFORMATION

Usage Limitation: The *EpiQuik*™ Histone Demethylase LSD1 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 LSD1 Activity/Inhibition Assay Kit and methods of use contain proprietary technologies by Epigentek. *EpiQuik*™ is a trademark of Epigentek Group Inc.

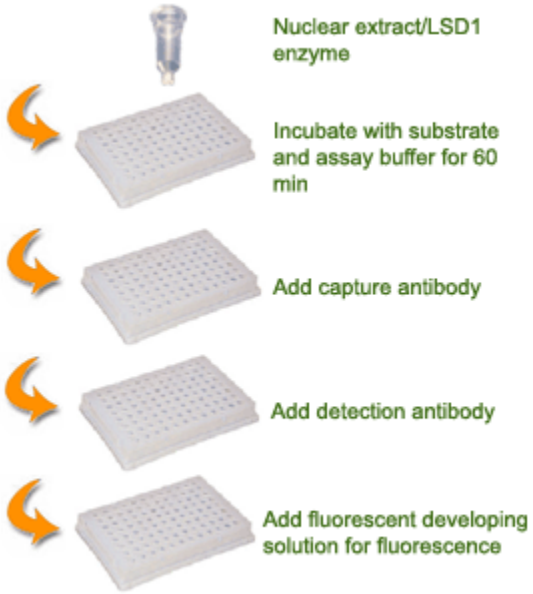
A BRIEF OVERVIEW

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. LSD1 histone 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. Currently, there are few methods available for measuring activity/inhibition of LSD1 using a variety of cells/tissues. The *EpiQuik*™ Histone Demethylase LSD1 Activity/Inhibition Assay Kit uses a proprietary and unique procedure to measure activity/inhibition of LSD1. 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 LSD1 activity and no interference by thiol-containing chemicals such as DTT, GSH, and 2-mercaptoethanol.
- Both cell/tissue extracts and purified LSD1 can be used, which allows the detection of inhibitory effects of LSD1 inhibitor *in vivo* and *in vitro*.
- Strip microplate format makes the assay flexible: manual or high throughput analysis.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The *EpiQuik*™ Histone Demethylase LSD1 Activity/Inhibition Assay Kit is designed for measuring cellular LSD1 activity/inhibition. In an assay with this kit, the unique di-methylated histone H3-K4 substrate is stably captured on the strip wells. Active LSD1 binds to and demethylates 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 LSD1 activity, can then be fluorometrically quantified.



Schematic Procedure for Using the *EpiQuik*™ Histone Demethylase LSD1 Activity/Inhibition Assay Kit

PROTOCOL

1. Prepare nuclear extracts from cells or tissues treated or untreated *in vivo* with LSD1 inhibitors 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). Dilute HG1 with distilled water (pH 7.2 to 7.5) at a 1:10 ratio (ex: 1 ml of HG1 + 9 ml of distilled water).

3. Dilute HG3 at a 1:30 ratio with HG2 (ex: 1 µl of HG3 + 29 µl of HG2). Add 28 µl of the diluted HG3, and 2 µl of *nuclear extract* (5-10 µg) or purified enzyme (amount to be optimized by end user) into each well.
   - For the no enzyme control, add 2 µl of HG2 instead of nuclear extract.
   - For the blank, add 30 µl of HG2 into the blank wells (no HG3 added).
   - For the standard curve, add 29 µl of HG2 into the wells (no HG3 added), followed by adding 1 µl of HG4 at different concentrations (0.1 – 10 ng/µl).
   - For *in vitro* LSD1 inhibition (in case of that all samples are untreated *in vivo*), add 2 µl of different amounts of tested inhibitors and reduce HG2 volume to 26 µl.

   Cover the wells with Parafilm M and incubate at room temperature for 45-60 minutes.

4. Aspirate and wash each well with 150 µl of diluted HG1 three times.

5. Dilute HG5 (at a 1:1000 ratio) to 1 µg/ml with diluted HG1. Add 50 µl of the diluted HG5 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 HG1** four times.

7. Dilute **HG6** (at a 1:1000 ratio) to 0.2 µg/ml with **diluted HG1**. Add 50 µl of the **diluted HG6** to each strip well and incubate at room temperature for 25-30 minutes.

8. Aspirate and wash each well with 150 µl of **diluted HG1** six times

9. Add 50 µl of **HG7** (Fluoro-Developer) into the wells and incubate at room temperature for 1-5 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. If the RFU reading is too high, dilute* **HG7** *with 1X PBS at a 1:5 ratio (ex: 10 µl* **HG7** *+ 40 µl of PBS), and then add the* **diluted HG7** *into the wells.*

10. Calculate LSD1 activity or inhibition. For simple calculation, use the following formulas:

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

$$\text{Inhibition \%} = \left(1 - \frac{\text{[RFU(control – blank) – RFU (treated sample – blank)]}}{\text{[RFU (control – blank) – RFU (untreated sample – blank)]}}\right) \text{x } 100\%$$

For an accurate calculation, plot Delta RFU value versus amount of **HG4** and determine the slope as delta RFU/ng.

Calculate LSD1 activity using the following formula:

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

TROUBLESHOOTING

No Signal for the Sample

The protein sample is not properly 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 frozen cells or tissues.	The nuclear extracts from frozen cells/tissue significantly lose enzyme activity. A fresh sample 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 order and if any steps of the procedure are omitted by mistake.
Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol are followed correctly.
Absence of LSD1 activity in 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 9 or dilute HG7 with PBS, and then add the diluted HG7 into the wells.

RELATED PRODUCTS

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