EpiQuik™ Histone Demethylase LSD1 Inhibitor Screening Assay Core Kit

Base Catalog # P-3075A

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiQuik™ Histone Demethylase LSD1 Inhibitor Screening Assay Core Kit is suitable for screening LSD1 inhibitors which directly interact with LSD1 or block the binding of LSD1 to its substrate.

Starting Material: The LSD1 enzyme should be a purified enzyme (active) with an LSD1 concentration of at least 200 ng per µl to achieve sufficient fluorescence intensity.

Internal Control: This kit includes an LSD1 Assay Standard, which is an oxidation product of LSD1 enzymatic reactions. This standard can be used as a control in quantifying oxidation product amounts generated from an LSD1 enzyme sample by comparing the fluorescence intensity of the sample with the standard.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the strip wells. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com

KIT CONTENTS

Component	48 Assays Cat. #P-3075A-48	96 Assays Cat. #P-3075A-96	Storage Upon Receipt
HH1 (LSD1 Assay Buffer)	2 ml	4 ml	4°C
HH2 (LSD1 Substrate, 0.7 mM)*	150 µl	300 µl	–20°C
HH3 (LSD1 Assay Standard, 100 mM)*	10 µl	20 µl	4°C
HH4 (LSD1 Inhibitor, 1 mM)*	25 µl	50 µl	4°C
HH5 (Fluoro Developer)*	12 µl	24 µl	–20°C
HH6 (Fluoro Enhancer)*	12 µl	24 µl	4°C
HH7 (Fluoro Diluter)*	4 ml	8 ml	4°C
8-Well Assay Strips (With Frame)	6	12	4°C
User Guide	1	1	RT

** Spin the solution down to the bottom prior to use.*

SHIPPING & STORAGE

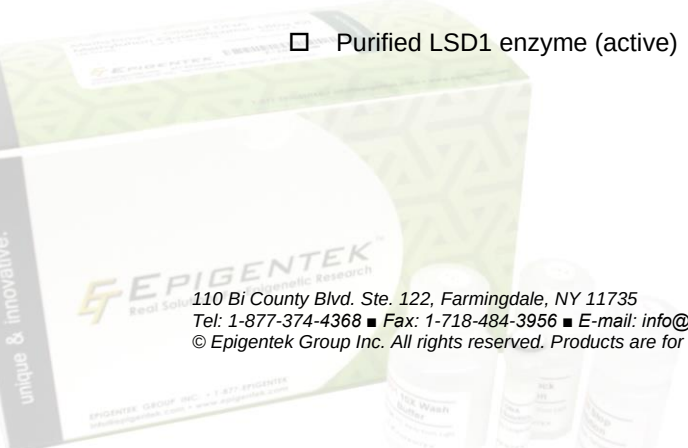
The kit is shipped in two parts: the first part at ambient room temperature and the second part on frozen ice packs at 4°C.

Upon receipt: (1) Store **HH2** and **HH5** at –20°C away from light; (2) Store remaining components (**HH1**, **HH3**, **HH4**, **HH6**, **HH7**, and **8-Well Assay Strips**) at 4°C away from light.

All components of the kit are stable for 6 months from the date of shipment, when stored properly.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Adjustable pipette
- ☐ Aerosol resistant pipette tips
- ☐ Microplate reader capable of reading fluorescence at 530 excitation and 590 emission
- ☐ 1.5 ml microcentrifuge tubes
- ☐ Water bath or Incubator for 37°C incubation
- ☐ Plate seal or Parafilm M
- ☐ Purified LSD1 enzyme (active)



GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the EpiQuik™ Histone Demethylase LSD1 Inhibitor Screening Assay Core Kit is tested against predetermined specifications to ensure consistent product quality. Epigentek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

Product Updates: Epigentek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Thus, only use the User Guide that was supplied with the kit when using that kit.

Usage Limitation: The EpiQuik™ Histone Demethylase LSD1 Inhibitor Screening Assay Core Kit is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The EpiQuik™ Histone Demethylase LSD1 Inhibitor Screening Assay Core Kit is part of proprietary technologies by Epigentek Group Inc.

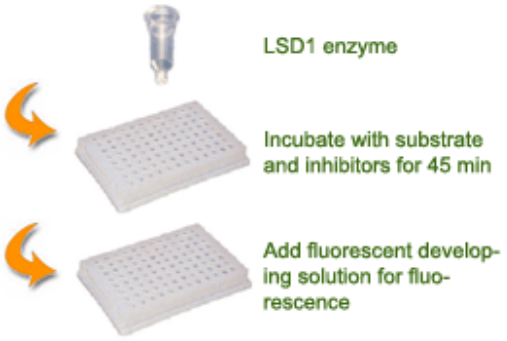
A BRIEF OVERVIEW

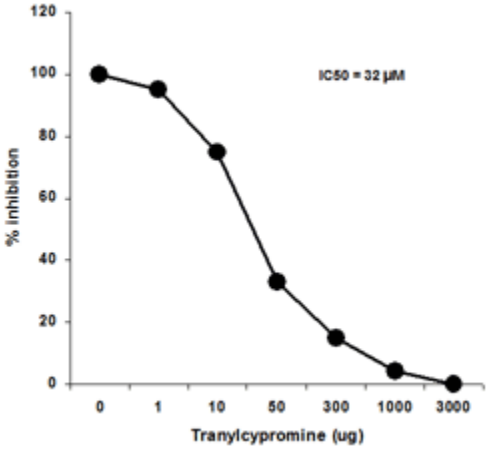
Lysine histone methylation is one of the most robust epigenetic marks and is essential for the regulation of multiple cellular processes. The methylation of H3-K4 seems to be of particular significance, as it is associated with active regions of the genome. H3-K4 methylation was considered irreversible 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 demethylase is also found to be involved in some pathological processes such as cancer progression. Inhibition of LSD1 may lead to re-methylation of H3-K4 and silencing of H3-K4 enriched active genes. The EpiQuik™ Histone Demethylase LSD1 Inhibitor Screening Assay Core Kit uses a unique procedure to measure inhibition of LSD1, with the following features:

- Fast procedure, which can be finished within 1 hour.
- Innovative homogeneous fluorescence assay without the need for radioactivity, extraction, or chromatography.
- Strip-based microplate format makes the assay flexible via manual or high throughput analysis.
- Simple, reliable, and consistent assay conditions

PRINCIPLE & PROCEDURE

The EpiQuik™ Histone Demethylase LSD1 Inhibitor Screening Assay Core Kit is designed for screening LSD1 demetylase inhibitors. In the assay with this kit, the unique di-methylated histone H3-K4 substrate is incubated with LSD1 in the strip wells. Active LSD1 binds to and demethylates histone H3-K4 substrate, producing hydrogen peroxide, which reacts with fluorogen 10-Acetyl-3,7-dihydroxy-phenoxazine and produces highly fluorescent oxidation products. The intensity of fluorescence from oxidation products is proportional to LSD1 enzyme activity. Therefore, as LSD1 activity decreases by inhibition, the fluorescence signal decreases.





Inhibition of LSD1 by tranylcypromine (TCP) LSD1 concentration: 500 ng/well

PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

Starting Materials

Input Enzyme: The LSD1 enzyme should be a purified enzyme (active) with an LSD1 concentration of at least 200 ng per µl to achieve sufficient fluorescence intensity.

1.

Predetermine the number of strip wells required for your experiment. Carefully remove un-needed strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C).

2.

Dilute your LSD1 enzyme with HH1 at an appropriate concentration of at least 200 ng per µl.

3.

Add the following components to the corresponding wells according to this chart:

Well Type	Component	Amount/Well
Control Wells	HH1	26 µl
	HH2	3 µl
	Diluted LSD1	1 µl
Standard Wells	HH1	26 µl
	HH2	3 µl
	HH3 (1-300 µM)	1 µl
Inhibitor Wells	HH1	23 µl
	HH2	3 µl
	Diluted LSD1	1 µl
	Inhibitor	3 µl
LSD1 Inhibitor Control Wells	HH1	23 µl
	HH2	3 µl
	Diluted LSD1	1 µl
	HH4	3 µl
Blank Wells	HH1	27 µl
	HH2	3 µl

Note: The inhibitor compound solution should not have thiol-containing chemicals such as DTT, GSH, and 2-mercaptoethanol, as the thiol-containing chemicals may interfere with the fluorometric determination. A standard curve can be generated by using different concentrations of HH3 (e.g., add 1 µl of LSD1 at 1, 3, 10, 30, 100, 300 µM to the standard wells).

4.

Mix and cover the strip wells with Parafilm M, and incubate at 37°C for 60 minutes.

5.

Prepare the **Fluorescence Development Solution** by adding 1 µl of **HH5** and 1 µl of **HH6** into each 400 µl of **HH7**.

6.

Add 50 µl of the **Fluorescence 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.

Use the following formula to calculate LSD1 activity. Plot RFU value versus amount of HH3 and determine the slope as delta RFU/µM.

$$LSD1\ Activity\ (\mu M/min/mg) = \frac{Untreated\ Sample\ RFU - Blank\ RFU}{Slope \times Minutes^{*} \times Amount\ of\ LSD1^{**}} \times 1000$$

* Incubation time used in Step 4.
** Amount of Diluted LSD1 in your control well used in Step 3.

Use the following formula to calculate LSD1 inhibition.

$$Inhibition\ \% = 1 - \left[\frac{Inhibitor\ Sample\ RFU - Blank\ RFU}{Control\ RFU - Blank\ RFU} \right] \times 100\%$$

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
No Signal for the No Inhibitor Control	Reagents are added incorrectly.	Check if reagents are added in the proper order and if any steps in the protocol 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.
	Insufficient input materials.	Ensure that a sufficient amount of enzyme (>200 ng) is added into the wells.
	Incorrect fluorescence reading.	Check if appropriate fluorescence filters ($530_{EX}/590_{EM}$) are used.
	Kit was not stored or handled properly.	Ensure all components of the kit were stored at the appropriate temperature and the cap is tightly capped after each opening or use.
No Inhibition by the Inhibitors	The amount of the inhibitors added is insufficient.	Ensure a sufficient amount of inhibitors is added into the reaction.
High background present in the negative control wells	Contaminated by the LSD1 enzyme.	Ensure the well is not contaminated from adding the enzyme accidentally or from using contaminated tips.

RELATED PRODUCTS

Histone Demethylase Assay

P-3074 EpiQuik™ Histone Demethylase (H3K4 Specific) Activity/Inhibition Assay Kit
P-3076 EpiQuik™ Histone Demethylase LSD1 Activity/Inhibition Assay Kit
P-3077 EpiQuik™ Histone Demethylase (H3K9 Specific) Activity/Inhibition Fast Assay Kit