ab113456 – Histone Demethylase KDM1/LSD1 Inhibitor Assay Kit

Instructions for Use

For screening KDM1/LSD1 inhibitors which directly interact with DM1/LSD1 or block the binding of KDM1/LSD1 to its substrate

This product is for research use only and is not intended for diagnostic use.

Version 2 Last Updated 20 February 2018

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1. BACKGROUND

Lysine histone methylation is one of the most robust epigenetic marks and is essential for the regulation of multiple cellular processes. The methylation of H3K4 seems to be of particular significance, as it is associated with active regions of the genome. H3K4 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 H3K4 specific histone demethylase, LSD1 and JARIDs, have been identified. LSD1 can remove di- and mono-methylation from H3K4 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 H3K4 and silencing of H3K4 enriched active genes.

ab113456 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.

Abcam's Histone Demethylase KDM1/LSD1 Inhibitor Assay Kit is designed for screening LSD1 demethylase inhibitors. In the assay with this kit, the unique di-methylated histone H3K4 substrate is incubated with LSD1 in the strip wells. Active LSD1 binds to and demethylates histone H3K4 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.

ab113456 is suitable for screening LSD1 inhibitors which directly interact with LSD1 or block the binding of LSD1 to its substrate.

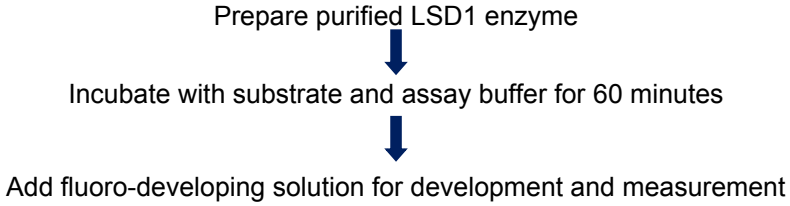
The LSD1 enzyme should be a purified enzyme (active) with an LSD1 concentration of at least 200 ng per µl to achieve sufficient fluorescence intensity.

ab113456 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.

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.

∆ Note: This kit may cross react with LSD2.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit as given in the table and away from light upon receipt.

Observe the storage conditions for individual prepared components in sections 9 & 10.

For maximum recovery of the products, centrifuge the original vial prior to opening the cap.

5. MATERIALS SUPPLIED

Item	48 Tests	96 Tests	Storage Condition (Before Preparation)
LSD1 Assay Buffer	2 mL	4 mL	4°C
LSD1 Substrate, 0.7 mM	150 µL	300 µL	-20°C
LSD1 Assay Standard, 100 mM	10 µL	20 µL	4°C
LSD1 Inhibitor, 1 mM	25µL	50 µL	4°C
Fluoro Developer	12 µL	24 µL	-20°C
Fluoro Enhancer	12 µL	24 µL	4°C
Fluoro Diluter	4 mL	8 mL	4°C
8-Well Assay Strip (with Frame)	6	12	4°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Adjustable pipette
- Aerosol resistant pipette tips
- Microplate reader capable of reading fluorescence at Ex/Em = 530/590 nm.
- 1.5 mL microcentrifuge tubes
- Water bath or Incubator for 37°C incubation
- Plate seal or Parafilm M
- Purified LSD1 enzyme (active)

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

All reagents provided are ready to use.

10. SAMPLE PREPARATION

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.

11. ASSAY PROCEDURE

11.1 Enzymatic Reaction

11.1.1 Predetermine the number of strip wells required for your experiment. It is advised to run replicate samples (include blank and positive control) to ensure that the signal generated is validated. 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).

11.1.2 Dilute your LSD1 enzyme with LSD1 Assay Buffer at an appropriate concentration of at least 200 ng per µL.

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

Well Type	Component	Amount/Well (µL)
Control Wells	LSD1 Assay Buffer	26
	LSD1 Substrate	3
	Diluted LSD1	1
Standard Wells	LSD1 Assay Buffer	26
	LSD1 Substrate	3
	LSD1 Assay Standard (1-300 µM)	1
Inhibitor Wells	LSD1 Assay Buffer	23
	LSD1 Substrate	3
	Diluted LSD1	1
	Inhibitor	3
LSD1 Inhibitor Control Wells	LSD1 Assay Buffer	23
	LSD1 Substrate	3
	Diluted LSD1	1
	LSD1 Inhibitor	3
Blank Wells	LSD1 Assay Buffer	27
	LSD1 Substrate	3

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 LSD1 Assay Standard (e.g. add 1 µl of LSD1 at 1, 3, 10, 30, 100, 300 µM to the standard wells).

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

11.2 **Signal Detection**

11.2.1 Prepare the Fluorescence Development Solution by adding 1 µL of Fluoro Developer and 1 µL of Fluoro Enhancer into each 400 µL of Fluoro Diluter.

11.2.2 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 Ex/Em = 530/590 nm.

Note: *If the stripwell microplate frame does not fit in the microplate reader, transfer the solution to a standard 96-well microplate.*

11.2.3 Calculate LSD1 activity or inhibition using the formulae provided in Section 12 – Data Analysis.

12. ANALYSIS

Plot RFU versus amount of LSD1 Assay Standard and determine the slope as delta RFU/µM then calculate LSD1 activity using the following formula:

$$\text{LSD1 activity (µM/min/mg)} = \frac{\text{Untreated Sample RFU – Blank RFU}}{\text{Slope x Incubation Time x Amount of LSD1**}} \times 1000$$

*Incubation time (minutes) at step 11.1.4.

**Amount of Diluted LSD1 in your control well used in step 11.1.3.

To calculated LSD1 inhibition:

$$\text{Inhibition \%} = 1 - \left(\frac{\text{Inhibitor Sample RFU – Blank RFU}}{\text{Control Sample RFU – Blank RFU}} \right) \times 100\%$$

13. TROUBLESHOOTING

Problem	Cause	Solution
No signal for the No Inhibitor Control	Reagents are added incorrectly	Check if reagents are added in the proper order with the right amount, 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 is 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 fluorescent wavelength (Ex/Em = 530/590 nm filter) is used
No signal for the No Inhibitor Control	Kit was not stored or handled properly	Ensure all components of the kit were stored at the appropriate temperature and caps are tightly capped after each opening or use
No Inhibition by the Inhibitors	The mount of the inhibitors added is insufficient	Ensure a sufficient amount of inhibitors is added to the reaction
High Background Present in the negative control wells	Contaminated by LSD1 enzyme	Ensure the well is not contaminated from adding the enzyme accidentally or from using contaminated tips

14. NOTES

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