



# Histone Demethylase LSD1 Activity/Inhibition Assay Kit

Catalog Number KA0575

96 assays

Version: 03

Intended for research use only

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## **Introduction**

### **Intended Use**

The 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 cell/ tissues, plants and bacteria.

### **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 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 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 of the Assay**

The 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.



## General Information

### Materials Supplied

List of components

Components	Quantity
HG1 (10X Wash Buffer)	30 mL
HG2 (LSD1 Assay Buffer)	4 mL
HG3 (LSD1 Substrate)*	100 µL
HG4 (LSD1 Assay Standard, 20 µg/mL)*	50 µL
HG5 (Capture Antibody 1000 µg/mL)*	10 µL
HG6 (Detection Antibody 200 µg/mL)*	16 µL
HG7 (Fluoro-developer)	12 mL
8-well Assay Strip (with Frame)	12 strips

\* For maximum recovery of the products, centrifuge the original vial after thawing prior to opening the cap.

### Storage Instruction

Upon receipt: (1) Store HG3, HG4, and HG6 at  $-20^{\circ}\text{C}$  away from light; (2) Store all other components (HG1, HG2, HG5, HG7, and 8-well Assay Strip) at  $4^{\circ}\text{C}$  away from light. The kit is stable for up to 6 months when stored properly.

*Note: Check if wash buffer, HG1, contains salt precipitates before using. If so, warm (at room temperature or  $37^{\circ}\text{C}$ ) and shake the buffer until the salts are re-dissolved.*

### Materials Required but Not Supplied

- ✓ Orbital shaker
- ✓ Pipettes and pipette tips
- ✓ Fluorescence microplate reader
- ✓ 1.5 mL microcentrifuge tubes
- ✓ 1X PBS

### Precautions for Use

- ✓ This Histone Demethylase LSD1 Activity/Inhibition Assay Kit is for research use only and is not intended for diagnostic and therapeutic application.
- ✓ Abnova guarantees the performance of all products in the manner described in our product instructions.

## Assay Protocol

### Assay Procedure

1. Prepare nuclear extracts from cells or tissues treated or untreated in vivo with LSD1 inhibitors by using your own successful method. Nuclear extracts can be used immediately or stored at  $-80^{\circ}\text{C}$  for future use.
2. Determine number of the 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  $4^{\circ}\text{C}$ ). Dilute HG1 with distilled water (pH 7.2-7.5) 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  $\mu\text{L}$  of HG3 + 29  $\mu\text{L}$  of HG2). Add 28  $\mu\text{L}$  of the diluted HG3, and 2  $\mu\text{L}$  nuclear extract (5-10  $\mu\text{g}$ ) or purified enzyme (amount to be optimized by end user) into each well.
  - For the no enzyme control, add 2  $\mu\text{L}$  of HG2 instead of nuclear extract.
  - For blank, add 30  $\mu\text{L}$  of HG2 into the blank wells (no HG3 added).
  - For standard curve, add 29  $\mu\text{L}$  of HG2 into the wells (no HG3 added), followed by adding 1  $\mu\text{L}$  of HG4 at different concentrations (0.1-10  $\text{ng}/\mu\text{L}$ ).
  - For in vitro LSD1 inhibition (in case of that all samples are untreated in vivo), add 2  $\mu\text{L}$  of different amounts of tested inhibitors and reduce HG2 volume to 26  $\mu\text{L}$ .

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

4. Aspirate and wash each well with 150  $\mu\text{L}$  of diluted HG1 three times.
5. Dilute HG5 (at a 1:1000 ratio) to 1  $\mu\text{g}/\text{mL}$  with diluted HG1. Add 50  $\mu\text{L}$  of the diluted HG5 to each strip well and incubate at room temperature for 60 min on an orbital shaker (50-100 rpm).
6. Aspirate and wash each well with 150  $\mu\text{L}$  of diluted HG1 four times.
7. Dilute HG6 (at a 1:1000 ratios) to 0.2  $\mu\text{g}/\text{mL}$  with diluted HG1. Add 50  $\mu\text{L}$  of the diluted HG6 to each strip well and incubate at room temperature for 25-30 min.
8. Aspirate and wash each well with 150  $\mu\text{L}$  of diluted HG1 six times.
9. Add 50  $\mu\text{L}$  of HG7 (Fluoro-Developer) into the wells and incubate at room temperature for 1-5 minutes away from light. Measure and read fluorescence on fluorescence microplate reader at  $530_{\text{EX}}/590_{\text{EM}}$  nm.

*Note: If the strip well frame does not fit the fluorescence reader, transfer the solution to the standard 96-well microplate and read fluorescence at  $530_{\text{EX}}/590_{\text{EM}}$  nm. If the RFU reading is too high, dilute the HG7 with 1X PBS at 1:5 ratio (ex: 10  $\mu\text{L}$  HG7 + 40  $\mu\text{L}$  of PBS) and then add the diluted HG7 into the wells.*

## Data Analysis

### Calculation of Results

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

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

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

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:

$$\text{Activity (ng/h/}\mu\text{g)} = \frac{\text{RFU}(\text{control} - \text{blank}) - \text{RFU}(\text{sample} - \text{blank})}{\text{slope} \times \text{h} \times \text{protein amount added } (\mu\text{g})}$$

## Resources

### 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 enough amount of protein.

The sample is not prepared from frozen cells or tissues.

The nuclear extracts from frozen cells/tissue significantly lost enzyme activity. The fresh sample should be used.

Nuclear extracts are stored incorrectly.

Ensure the nuclear extracts are stored at  $-80^{\circ}\text{C}$ .

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 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 9 or dilute HG7 with PBS, and then add the diluted HG7 into the wells.

**Plate Layout**

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	A	B	C	D	E	F	G	H