Instructions for Use

For the measurement of activity/inhibition of KDM1/LSD1 histone demethylase using cell/tissue extracts

This product is for research use only and is not intended for diagnostic use.
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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 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 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 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 H3K4 and silencing of H3K4 enriched active genes. Currently, there are few methods available for measuring activity/inhibition of LSD1 using a variety of cells/tissues.

ab113457 uses a proprietary and unique procedure to measure activity/inhibition of LSD1.

This Kit has the following advantages:

- 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
INTRODUCTION

The Histone Demethylase KDM1/LSD1 Activity Quantification 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 H3K4 substrate. The remaining un-demethylated substrate can be recognized with a high affinity anti-methylated histone H3K4 antibody. The ratio or amount of the un-demethylated histone, which is inversely proportional to LSD1 activity, can then be fluorometrically quantified.

2. ASSAY SUMMARY

Nuclear Extract/LSD1 enzyme

↓

Incubate with substrate and assay buffer

↓

Wash wells, then add capture antibody

↓

Wash wells, then add detection antibody

↓

Add developing solution for color development, then measure
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 upon receipt away from light.

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.

Check if 10X Wash Buffer contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are redissolved.
5. MATERIALS SUPPLIED

<table>
<thead>
<tr>
<th>Item</th>
<th>48 Tests</th>
<th>96 Tests</th>
<th>Storage Condition (Before Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X Wash Buffer</td>
<td>15 mL</td>
<td>30 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>LSD1 Assay Buffer</td>
<td>2 mL</td>
<td>4 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>LSD1 Substrate*</td>
<td>50 µL</td>
<td>100 µL</td>
<td>-20°C</td>
</tr>
<tr>
<td>LSD1 Standard, 20 µg/mL*</td>
<td>25 µL</td>
<td>50 µL</td>
<td>-20°C</td>
</tr>
<tr>
<td>Capture Antibody 1000 µg/mL*</td>
<td>5 µL</td>
<td>10 µL</td>
<td>4°C</td>
</tr>
<tr>
<td>Detection Antibody 200 µg/mL*</td>
<td>8 µL</td>
<td>16 µL</td>
<td>-20°C</td>
</tr>
<tr>
<td>Fluoro-Developer</td>
<td>6 mL</td>
<td>12 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>8-Well Assay Strip (with Frame)</td>
<td>6</td>
<td>12</td>
<td>4°C</td>
</tr>
</tbody>
</table>

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

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Adjustable pipette or multiple-channel pipette
- Multiple-channel pipette reservoirs
- Aerosol resistant pipette tips
- Fluorescent microplate reader
- 1.5 mL microcentrifuge tubes
- Incubator for 37°C incubation
- Distilled water
- Parafilm M or aluminium foil
- PBS
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

Prepare fresh reagents immediately prior to use.

9.1 **1X Wash Buffer**

Dilute 10X Wash Buffer with distilled water (pH 7.2 to 7.5) at a 1:10 ratio (ex: 1 mL of 10X Wash Buffer + 9 mL of distilled water). This 1X Wash Buffer can now be stored at 4°C for up to six months.

9.2 **Diluted Capture Antibody Solution**

Prepare Diluted Capture Antibody Solution: Dilute Capture Antibody with 1X Wash Buffer at a ratio of 1:1000 (i.e., add 1 µL of Capture Antibody to 1000 µL of 1X Wash Buffer). 50 µL of Diluted Capture Antibody will be required for each assay well.

9.3 **Diluted Detection Antibody Solution**

Prepare Diluted Detection Antibody Solution: Dilute Detection Antibody with 1X Wash Buffer at a ratio of 1:2000 (i.e., add 1 µL of Detection Antibody to 2000 µL of 1X Wash Buffer). 50 µL of this Diluted Detection Antibody will be required for each assay well.

9.4 **LSD1 Substrate Solution**

Dilute LSD1 Substrate at a 1:30 ratio with Assay Buffer, (ex: 1 µL of LSD1 Substrate + 29 µL of Assay Buffer)

9.5 **Diluted Assay Standard Solution**

Suggested Standard Curve Preparation: dilute Assay Standard with Antibody Buffer to cover range 0.1-10 ng/mL.

*Note:* Keep each of the diluted solutions (except 1X Wash Buffer) on ice until use. Any remaining diluted solutions, other than 1X Wash Buffer, should be discarded if not used within the same day.
10. **SAMPLE PREPARATION**

Prepare nuclear extracts by using your own successful method. For your convenience and the best results, Abcam offers a nuclear extraction kit (ab113474) optimized for use in series Protein Preparation.

Nuclear extracts can be used immediately or stored at –80°C for future use.
11. ASSAY PROCEDURE

11.1 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).

11.2 Add 28 µL of the diluted LSD1 Substrate, 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 Assay Buffer instead of nuclear extract.
For the blank, add 30 µL of Assay Buffer into the blank wells (no diluted LSD1 Substrate added).
For the standard curve, add 29 µL of Assay Buffer into the wells (no diluted LSD1 Substrate added), followed by adding 1 µL of LSD1 Standard at different concentrations (0.1 - 10 ng/ µL).

11.3 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 Assay Buffer volume to 26 µL.

11.4 Cover the wells with Parafilm M and incubate at room temperature for 30-45 minutes.

11.5 Aspirate and wash each well with 150 µL of 1X Wash Buffer two times.

11.6 Add 50 µL of the diluted Capture Antibody to each strip well and incubate at room temperature for 60 minutes on an orbital shaker (50-100 rpm).

11.7 Aspirate and wash each well with 150 µL of 1X Wash Buffer four times.

11.8 Add 50 µL of the diluted Detection Antibody to each strip well and incubate at room temperature for 25-30 min.

11.9 Aspirate and wash each well with 150 µL of 1X Wash Buffer five to six times.

11.10 Add 50 µL of the 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 Ex/Em = 530/590nm.
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 Ex/Em = 530/590. If the RFU reading is too high, dilute Fluoro-Developer with 1X PBS at a 1:5 ratio (ex: 10 µL Fluoro-Developer + 40 µL of PBS), and then add the diluted Fluoro-Developer into the wells.
12. **ANALYSIS**

Calculate LSD1 activity or inhibition.

For simple calculation use the following formulae:

\[
\text{LSD1 activity (RFU/h/\mu g)} = \frac{[\text{RFU (control – blank)} – \text{RFU (untreated sample – blank)}]}{\text{reaction time (h) \times protein amount added (\mu g)}}
\]

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

For an accurate calculation, plot Delta RFU value versus amount of LSD1 Standard and determine the slope as delta RFU/ng.

Calculate LSD1 (H3K4) activity using the following formula:

\[
\text{Activity (ng/h/\mu g)} = \frac{[\text{RFU (control-blank)} – \text{RFU (sample – blank)}]}{\text{slope \times reaction time (h) \times protein amount added (\mu g)}}
\]
## 13. TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Signal for the Sample</td>
<td>The protein sample is not properly extracted</td>
<td>Ensure the protein extraction protocol is suitable for nuclear protein extraction</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The protein amount is added into well insufficiently</td>
</tr>
<tr>
<td></td>
<td>The sample is prepared from frozen cells or tissues</td>
<td>The nuclear extracts from frozen cells/tissue significantly lose enzyme activity. A fresh sample should be used</td>
</tr>
<tr>
<td></td>
<td>Nuclear extracts are incorrectly stored</td>
<td>Ensure the nuclear extracts are stored at –80°C</td>
</tr>
<tr>
<td></td>
<td>Reagents are added incorrectly</td>
<td>Check if reagents are added in order and if any steps of the procedure have been omitted by mistake</td>
</tr>
<tr>
<td></td>
<td>Incubation time and temperature is incorrect</td>
<td>Ensure the incubation time and temperature described in the protocol is followed correctly</td>
</tr>
<tr>
<td>No Signal for the Sample</td>
<td>Absence of LSD1 activity in the sample due to treatment</td>
<td>N/A</td>
</tr>
<tr>
<td>High Background Present for the Blank</td>
<td>The well is not washed sufficiently</td>
<td>Check if wash at each step is performed according to the protocol</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>--------------------------------------</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Overdevelopment</td>
<td>Decrease development time or dilute Fluoro-Developer with PBS, and then add the diluted Fluoro-Developer into the wells</td>
<td></td>
</tr>
</tbody>
</table>
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