

ab113460 – KDM1/LSD1 Activity Quantification Kit (Fluorometric)

Instructions for Use

For the measurement of activity/inhibition of KDM1/LSD1 using nuclear extracts or purified enzymes from a broad range of species

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 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 (BHC110, KDM1) and JARIDs have been identified. LSD1 can remove di- and mono-methylation from H3K4 by using an amine oxidase reaction. LSD1 is associated with complexes that function as both transcriptional inactivators and activators. It demethylates mono-/di-methyl H3K4 when associated with the Co-REST complex at neuronal genes, or mono-/di-methyl H3K9 when associated with the androgen receptor.

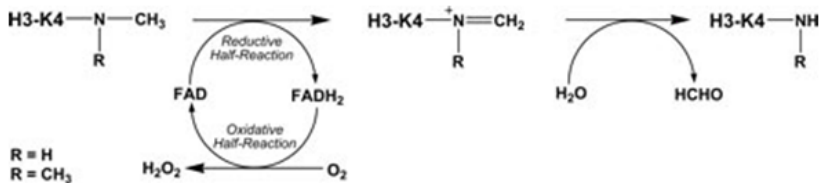


Figure 1. Histone H3K4 demethylation reaction catalyzed by LSD1

LSD1 is found to be pivotal in development and differentiation. For example, this enzyme is required to induce skeletal muscle differentiation, and mutation of drosophila LSD1 results in tissue-specific defect in development through disrupting H3K4 methylation. LSD1 is also shown to participate in regulation of chromatin remodeling, cell death and global DNA methylation. More importantly, LSD1 is found to be involved in some pathological processes such as cancer and inflammatory diseases. For example, expression of LSD1 is observed in cancer and LSD1 triggers MYC and E2F-mediated transcription in cancer cells. Detection of activity and inhibition of

LSD1 would be important in elucidating mechanisms of epigenetic regulation of gene activation and silencing and benefiting cancer diagnostics and therapeutics.

There are only a couple of methods used for detecting LSD1 activity/inhibition. These methods are based on the measurement of H_2O_2 or formaldehyde release, a by-product of LSD1 enzymatic reaction and have significant weaknesses including: (1) Large amount (at μg level) of substrate and enzyme are required; (2) Nuclear extracts from cell/tissues cannot be used; (3) Redox-sensitive LSD1 inhibitors are not suitable for testing with these methods; (4) Highly interfered by DMSO and thiol-containing chemicals, which are often contained in enzyme solution or tested compound solvents; and (5) Less accuracy than direct measurement of LSD1-converted demethylated product. These problems were averted with the earlier ab113457, a popular assay method for LSD1 activity/inhibition. We have now added an improved version, ab113460 retains the simplicity, rapidness, high throughput, and non-radioactivity featured in the previous version, and has the following advantages:

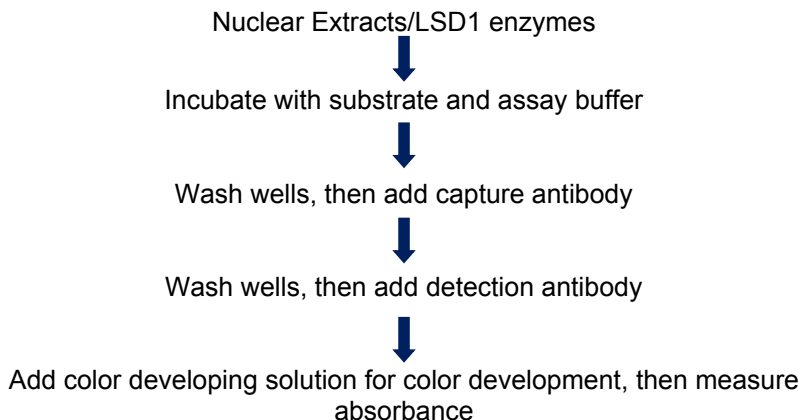
- Strip-well microplate format makes the assay flexible and quick: manual or high throughput analysis that can be completed within 3 hours
- Enhanced kit composition enables background signals to be extremely low, which allows the assay to be more accurate, sensitive, reliable, and consistent.
- Innovative fluorometric assay directly measures LSD1 activity by a straightforward detection of LSD1-converted demethylated product, rather than by-products. Thus it eliminates assay interferences caused by thiol-containing chemicals such as DTT, GSH, and 2-mercaptoethanol
- Both cell/tissue extracts and purified LSD1 can be used, which allows for the detection of inhibitory effects of LSD1 inhibitor in vivo and in vitro
- Novel assay principle allows high sensitivity to be achieved. The activity can be detected from as low as 2 ng of purified LSD1 enzyme, which is about 50 fold higher than that obtained by H_2O_2 /formaldehyde release-based LSD1 assays

- Demethylated H3K4 standard is included, which allows the specific activity of LSD1 to be quantified

The KDM1/LSD1 Activity Quantification Assay Kit (Fluorometric) contains all reagents necessary for the measurement of KDM1/LSD1 activity/inhibition. In this assay, di-methylated histone H3K4 LSD1 substrate is stably coated onto the strip wells. Active LSD1 binds to the substrate and removes methyl groups from the substrate. The LSD1-demethylated products can be recognized with a specific antibody. The ratio or amount of demethylated products, which is proportional to enzyme activity, can then be fluorometrically measured by reading the fluorescence in a fluorescence microplate reader. The activity of LSD1 enzyme is proportional to the fluorescent intensity measured. ab113460 is suitable for measuring LSD1 activity/inhibition using nuclear extracts or purified enzymes from a broad range of species such as mammals, plants, fungi, and bacteria, in a variety of forms including cultured cells and fresh tissues.

Δ 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 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 the 10X Wash Buffer contains salt precipitates before use. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved.

5. MATERIALS SUPPLIED

Item	48 Tests	96 Tests	Storage Condition (Before Preparation)
10X Wash Buffer	14 mL	28 mL	4°C
LSD1 Assay Buffer	4 mL	8 mL	RT
LSD1 Substrate, 50 µg/mL	60 µL	120 µL	-20°C
LSD1 Assay Standard, 50 µg/mL	10 µL	20 µL	-20°C
Capture Antibody, 1000 µg/mL	5 µL	10 µL	4°C
Detection Antibody, 400 µg/mL	6 µL	12 µL	-20°C
LSD1 Inhibitor Tranylcypromine, 1 mM	20 µL	40 µL	4°C
Fluoro Developer*	10 µL	20 µL	-20°C
Fluoro Enhancer*	10 µL	20 µL	4°C
Fluoro Diluter*	4 mL	8 mL	RT
8-Well Assay Strip (with Frame)	6	12	4°C
Adhesive Covering Film	1	1	RT

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
- Nuclear extract or purified enzymes
- Parafilm M or aluminium foil

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
- The LSD1 assay standard (demethylated histone H3K4) is provided in this kit for quantification of LSD1 enzyme activity. Because LSD1 activity can vary from tissue to tissue, and from normal and diseased states, it is advised to run replicate samples to ensure that the signal generated is validated
- **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 the 10X Wash Buffer with distilled water (pH 7.2 to 7.5) at a 1:10 ratio (e.g. 13 mL of 10X Wash Buffer + 117 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

Dilute the Capture Antibody with 1X Wash Buffer at a ratio of 1:1000 (e.g. add 1 µL of Capture Antibody to 1000 µL of 1X Wash Buffer). 50 µL of the 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 Fluorescence Developer Solution

Add 1 µL of Fluoro Developer and 1 µL of Fluoro Enhancer to every 500 µL of Fluoro Diluter.

9.5 Diluted Assay Standard Solution

First, dilute Assay Standard with Assay Buffer to 5 ng/µL by adding 1 µL of Assay Standard to 9 µL of Assay Buffer. Then, further prepare five concentrations by combining the 5 ng/µL diluted Assay Standard with Assay Buffer into final concentrations of 0.2, 0.5, 1, 2, and 5 ng/µL according to the following dilution chart:

ASSAY PREPARATION

Tube	5ng/ μ L Assay Standard (μ L)	Assay Buffer (μ L)	Resulting Assay Standard concentration (ng/ μ L)
1	1.0	24.0	0.2
2	1.0	9.0	0.5
3	1.0	4.0	1.0
4	2.0	3.0	2.0
5	4.0	0.0	5.0

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.

9.6 Suggested Working Buffer and Solution Setup

The table below shows the approximate amount of required buffers and solutions for defined assay wells, based on the protocol.

Reagents	1 well	8 wells (1 strip)	16 wells (2 strips)	48 wells (6 strips)
1X Wash Buffer	2.5 mL	20 mL	40 mL	120 mL
Assay Buffer	50 μ L	400 μ L	800 μ L	2400 μ L
LSD1 Substrate	1 μ L	8 μ L	16 μ L	50 μ L
Assay Standard	NA	NA	1 μ L (optional)	2 μ L
Capture Antibody Solution	50 μ L	400 μ L	800 μ L	2400 μ L
Detection Antibody	50 μ L	400 μ L	800 μ L	2400 μ L
Fluorescence Development Solution	0.05mL	0.4mL	0.8 mL	2.4 mL

10. SAMPLE PREPARATION

ab113460 is suitable for measuring LSD1 activity/inhibition using nuclear extracts or purified enzymes from a broad range of species such as mammals, plants, fungi, and bacteria, in a variety of forms including cultured cells and fresh tissues. Nuclear extracts can be prepared by using your own successful method. For your convenience and the best results, Abcam offers a nuclear extraction kit (ab113474) that is optimized for use with this kit.

Purified enzymes can be active LSD1 from recombinant proteins or isolated from cell/tissues.

Input materials can be nuclear extracts or purified LSD1 enzymes. The amount of nuclear extracts for each assay can be 0.5 µg - 20 µg with an optimal range of 5 - 10 µg. The amount of purified enzymes can be 5 ng - 500 ng, depending on the purity and catalytic activity of the enzymes.

Nuclear extracts can be used immediately or stored at -80°C for future use.

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 Blank Wells: Add 49 μL of LSD1 Assay Buffer and 1 μL of Substrate to each blank well.

Standard Wells: For a standard curve, add 49 μL of Assay Buffer and 1 μL of Diluted Assay Standard solution to each standard well with a minimum of five wells, each at a different concentration between 0.2 - 5 $\text{ng}/\mu\text{L}$ (based on the dilution chart in Section 9.5).

Sample Wells Without Inhibitor: Add 45 μL - 48 μL Assay Buffer, 1 μL of Substrate, and 1 - 4 μL of your nuclear extracts or 1 - 4 μL of your purified LSD1 enzyme to each sample well without inhibitor. Total volume should be 50 μL per well.

Sample Wells with Inhibitor: Add 40 μL - 43 μL of Assay Buffer, 1 μL of Substrate, 1 to 4 μL of your nuclear extracts or 1 to 4 μL of your purified LSD1 enzyme, and 5 μL of inhibitor solution. Total volume should be 50 μL per well.

Note: Follow the suggested well setup diagram below. It is recommended to use 2 μg – 10 μg of nuclear extract per well or 10 ng – 100 ng of purified enzyme per well. The concentration of inhibitors to be added into the sample wells can be varied (e.g., 1 μM – 1000 μM). However, the final concentration of the inhibitors before adding to the wells should be prepared with Assay Buffer at a 1:10 ratio (e.g., add 0.5 μL of inhibitor to 4.5 μL of Assay Buffer), so that the original solvent of the inhibitor can be reduced to 1% of the reaction solution or less. The Jumonji demethylase general inhibitor, N-Oxalylglycine can be used as a control inhibitor.

11.1.3 Tightly cover the strip-well microplate with the Adhesive Covering Film to avoid evaporation, and incubate at 37°C for 60 - 120 min.

Note: *The incubation time may depend on intrinsic LSD1 activity. In general, 60-90 min incubation is suitable for active purified LSD1 enzymes and 90-120 min incubation is required for nuclear extracts. The Adhesive Covering Film can be cut to the required size to cover the strips based on the number of strips to be used.*

11.1.4 Remove the reaction solution from each well. Wash each well with 150 µL of the 1X Wash Buffer each time for three times.

11.2 Antibody Binding & Signal Enhancing

11.2.1 Add 50 µL of the Diluted Capture Antibody to each well, then carefully cover with Parafilm M or aluminium foil and incubate at room temperature for 60 min.

11.2.2 Remove the Diluted Capture Antibody solution from each well.

11.2.3 Wash each well with 150 µL of the 1X Wash Buffer each time for three times.

11.2.4 Add 50 µL of the Diluted Detection Antibody to each well, then carefully cover with Parafilm M or aluminium foil and incubate at room temperature for 30 min.

11.2.5 Remove the Diluted Detection Antibody solution from each well.

11.2.6 Wash each well with 150 µL of the 1X Wash Buffer each time for four times.

Note: *Ensure any residual wash buffer in the wells is thoroughly removed as much as possible at each wash step.*

11.3 Signal Detection

11.3.1 Add 50 µL of Fluorescence Development Solution to each well and incubate at room temperature for 2 - 4 min away from direct light. The Fluorescence Development Solution will turn pink in the presence of sufficient demethylated products.

11.3.2 Read the fluorescence on a fluorescence microplate reader within 2 to 10 min at Ex/Em = 530/590 nm.

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

ASSAY PROCEDURE

Suggested Strip Well Setup: The suggested strip-well plate setup for the LSD1 activity assay in a 48-assay format is shown in the table below. The controls and samples can be measured in duplicates

Well #	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
A	Blank	Blank	Sample	Sample	Sample	Sample
B	Assay Standard 0.2 ng	Assay Standard 0.2 ng	Sample	Sample	Sample	Sample
C	Assay Standard 0.5 ng	Assay Standard 0.5 ng	Sample	Sample	Sample	Sample
D	Assay Standard 1.0 ng	Assay Standard 1.0 ng	Sample	Sample	Sample	Sample
E	Assay Standard 2.0 ng	Assay Standard 2.0 ng	Sample	Sample	Sample	Sample
F	Assay Standard 5.0 ng	Assay Standard 5.0 ng	Sample	Sample	Sample	Sample
G	Sample	Sample	Sample	Sample	Sample	Sample
H	Sample	Sample	Sample	Sample	Sample	Sample

ASSAY PROCEDURE

Single Standard Control Setup.

The suggested strip-well plate setup for the LSD1 activity assay in a 48-assay format.

Well #	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
A	Blank	Blank	Sample	Sample	Sample	Sample
B	Standard 1.0 ng	Standard 1.0 ng	Sample	Sample	Sample	Sample
C	Sample	Sample	Sample	Sample	Sample	Sample
D	Sample	Sample	Sample	Sample	Sample	Sample
E	Sample	Sample	Sample	Sample	Sample	Sample
F	Sample	Sample	Sample	Sample	Sample	Sample
G	Sample	Sample	Sample	Sample	Sample	Sample
H	Sample	Sample	Sample	Sample	Sample	Sample

12. ANALYSIS

Calculate LSD1 activity or inhibition.

Calculate average duplicate readings for sample wells and blank wells.

For simple calculation with a single Standard Control use the following formula:

$$\text{LSD1 activity (RFU/min/mg)} = \frac{(\text{Sample RFU} - \text{Blank RFU})}{(\text{Protein Amount } (\mu\text{g})^* \times \text{min}^{**})} \times 1000$$

* Protein amount (μg) added into the reaction at section 11.1.2 in μg .

** Incubation time (minutes) at section 11.1.3

Example calculation:

Average RFU of sample is 6800

Average RFU of blank is 800

Protein amount is 5 μg

Incubation time is 2 hours (120 min)

$$\begin{aligned}\text{LSD1 activity} &= [(6800 - 800) / (5 \times 120)] \times 1000 \\ &= 10000 \text{ RFU/min/mg}\end{aligned}$$

For accurate or specific activity calculation:

Generate a standard curve and plot RFU value versus amount of LSD1 Standard at each concentration point.

Determine the slope as RFU/ng (you can use Microsoft Excel statistical functions for slope calculation), then calculate the amount of LSD1-converted demethylated product using the following formulae:

$$\text{Demethylated Product (ng)} = \frac{(\text{Sample RFU} - \text{Blank RFU})}{\text{Slope}}$$

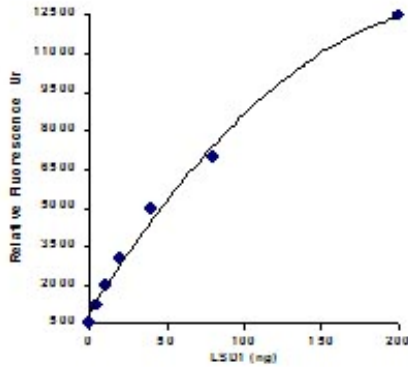
$$\text{LSD1 activity (ng/min/mg)} = \frac{\text{Demethylated Product (ng)}}{(\text{Protein Amount } (\mu\text{g}) \times \text{min}^*)} \times 1000$$

* Incubation time (minutes) at section 11.1.3

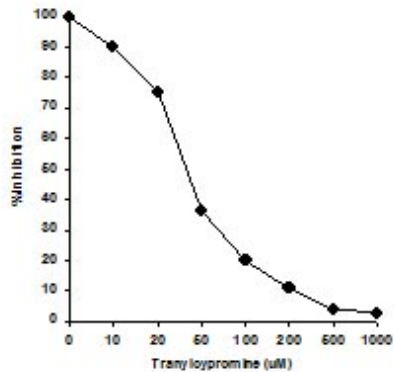
For inhibition calculation:

$$\text{Inhibition \%} = 1 - \left(\frac{\text{Inhibitor Sample RFU} - \text{Blank RFU}}{\text{No inhibitor sample RFU} - \text{Blank RFU}} \right) \times 100\%$$

Typical Data Graphs:



Demonstration of high sensitivity of KDM1/LSD1 activity assay achieved by using recombinant KDM1/LSD1 with ab113460.



Demonstration of inhibitory effects of a KDM1/LSD1 inhibitor detected by ab113460.

13. TROUBLESHOOTING

Problem	Cause	Solution
No signal or weak signal in both the positive control and sample wells.	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
	The well is incorrectly washed before enzyme reaction	Ensure the well is not washed prior to adding the positive control and sample
	Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol is followed correctly
	Incorrect fluorescence reading	Check if appropriate fluorescent wavelength (Ex/Em = 530/590 nm filter) is used
No signal or weak signal in both the positive control and sample wells.	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 signal or weak signal in only the standard curve wells	The standard amount is insufficiently added to the well in 11.1.2	Ensure a sufficient amount of standard is added
	The standard is degraded due to improper storage conditions	Follow the Storage guidance for storage instructions of LSD1 Assay Standard.
High Background Present for the Blank	Insufficient washing of wells	Check if washing recommendations at each step is performed according to the protocol

RESOURCES

Problem	Cause	Solution
	Contaminated by sample or positive control.	Ensure the well is not contaminated from adding sample or standard accidentally or from using contaminated tips
High Background Present for the Blank	Incubation time with detection antibody is too long	The incubation time at 11.2.4 should not exceed 45 min
	Over development of color.	Decrease the development time in 11.3.1 and measure absorbance as quickly as possible
No signal or weak signal only in sample wells	Protein sample is not properly extracted or purified	Ensure your protocol is suitable for LSD1 protein extraction. For the best results, it is advised to use Abcam's Nuclear Extraction Kit (ab113474). Also, use fresh cells or tissues for protein extraction, as frozen cells or tissues could lose enzyme activity

14. NOTES

RESOURCES

RESOURCES

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