CREATIVE BIOMART®
Assay Kit

LSD1 Demethylase Activity/Inhibition Fluorometric Assay Kit

Product Information

Common Name

LSD1 Demethylase

Cat.No. Kit-0524

Product Overview

LSD1 Demethylase Activity/Inhibition Assay Kit (Fluorometric) is used for an easy and fast fluorometric measurement of LSD1 activity or inhibition.

Description

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 (BHC110, KDM1) and JARIDs have been identified. LSD1 can remove di- and mono-methylation from H3-K4 by using an amine oxidase reaction. LSD1 is associated with complexes that function as both transcriptional inactivators and activators. It demethylates mono-/di-methyl H3-K4 when associated with the Co-REST complex at neuronal genes, or mono-/di-methyl H3-K9 when associated with the androgen receptor.

Applications

The LSD1 Demethylase Activity/Inhibition Assay Kit (Fluorometric) 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. Nuclear extracts can be used immediately or stored at -80°C for future use. Purified enzymes can be active LSD1 from recombinant proteins or isolated from cell/tissues.

Usage

For research use only (RUO)

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Storage

Upon receipt: (1) Store LF3, LF4, LF6 and LF8 at –20°C away from light; (2) Store LF1, LF5, LF7, LF9, and 8-Well Assay Strips at 4°C away from light; (3) Store remaining components (LF2, LF10, and Adhesive Covering Film) at room temperature away from light.
All components of the kit are stable for 6 months from the date of shipment, when stored properly.
Note: (1) Check if LF1 (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.

Kit Components

LF1 (10X Wash Buffer) 14 mL
LF2 (LSD1 Assay Buffer) 4 mL
LF3 (LSD1 Substrate, 50 µg/mL)* 60 µL
LF4 (LSD1 Assay Standard, 50 µg/mL)* 10 µL
LF5 (Capture Antibody, 1000 µg/mL*) 5 µL
LF6 (Detection Antibody, 400 µg/mL)* 6 µL
LF7 (LSD1 Inhibitor Tranylcypromine, 1 mM)* 20 µL
LF8 (Fluoro Developer) 10 µL
LF9 (Fluoro Enhancer) 10 µL
LF10 (Fluoro Diluter) 4 mL
8-Well Assay Strips (With Frame) 6 strips
Adhesive Covering Film 1 slice
* Spin the solution down to the bottom prior to use.

Materials Required but Not Supplied

Adjustable pipette or multiple-channel pipette
Multiple-channel pipette reservoirs
Aerosol resistant pipette tips
Fluorescence microplate reader capable of reading fluorescence at 530ex/590em nm
1.5 mL microcentrifuge tubes
Incubator for 37°C incubation

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Distilled water
Nuclear extract or purified enzymes
Parafilm M or aluminum foil

Detection method Fluorometric

Features & Benefits

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 H2O2/formaldehyde release-based LSD1 assays.
Demethylated H3-K4 standard is included, which allows the specific activity of LSD1 to be quantified.

Preparation

Prepare Diluted LF1 1X Wash Buffer: Add 13 mL of LF1 10X Wash Buffer to 117 mL of distilled water and adjust pH to 7.2-7.5. This Diluted LF1 1X Wash Buffer can now be stored at 4°C for up to six months.
Prepare Diluted LF5 Capture Antibody Solution: Dilute LF5 Capture Antibody with Diluted LF1 1X Wash Buffer at a ratio of 1:1000 (add 1 µL of LF5 to 1000 µL of Diluted LF1 1X Wash Buffer). 50 µL of Diluted LF5 will be required for each assay well.
Prepare Diluted LF6 Detection Antibody Solution: Dilute LF6 Detection Antibody with Diluted LF1 1X Wash Buffer at a ratio of 1:2000 (add 1 µL of LF6 Detection Antibody to 2000 µL of Diluted LF1 1X

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Wash Buffer). 50 µL of Diluted LF6 will be required for each assay well.
Prepare Diluted LF4 Assay Standard Solution: Suggested Standard Curve Preparation: First, dilute LF4 with LF2 to 5 ng/µL by adding 1 µL of LF4 to 9 µL of LF2. Then, further prepare five concentrations by combining the 5 ng/µL diluted LF4 with LF2 into final concentrations of 0.2, 0.5, 1, 2, and 5 ng/µL according to the following dilution chart:
Tube LF4 (5 ng/µL) LF2 Resulting LF4 Concentration
1 1.0 µL 25.0 µL 0.2 ng/µL
2 1.0 µL 9.0 µL 0.5 ng/µL
3 1.0 µL 4.0 µL 1.0 ng/µL
4 2.0 µL 3.0 µL 2.0 ng/µL
5 4.0 µL 0.0 µL 5.0 ng/µL
Prepare Fluorescence Development Solution: Add 1 µL of LF8 Fluoro Developer and 1 µL of LF9 Fluoro Enhancer to every 500 µL of LF10 Fluoro Diluter.
Note: Keep each of diluted solutions except Diluted LD1 1X Wash Buffer on ice until use. Any remaining diluted solutions other than Diluted LD1 should be discarded if not used within the same day.

Assay Protocol

Input Amount: The amount of nuclear extracts for each assay can be 0.5 µg to 20 µg with optimized range of 5-10 µg. The amount of purified enzymes can be 2 ng to 500 ng, depending on the purity and catalytic activity of the enzymes.
Nuclear Extraction: You can use your method of choice for preparing nuclear extracts.
Nuclear Extract or Purified LSD1 Storage: Nuclear extract or purified LSD1 enzyme should be stored in aliquots at –80°C until use.
Enzymatic Reaction
1. Predetermine the number of strip wells required for your experiment. It is advised to run replicate samples (include blank and standard controls) 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).
2. Blank Wells: Add 49 µL of LF2 and 1 µL of LF3 to each blank well.

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3. Standard Wells: For a standard curve, add 49 µL of LF2 and 1 µL of Diluted LF4 standard solution to each standard well with a minimum of five wells, each at a different concentration between 0.2 to 5 ng/µL (based on the dilution chart in Reagent Preparation).
4. Sample Wells Without Inhibitor: Add 44 to 48 µL of LF2, 1 µL of LF3, and 1 to 5 µL of your nuclear extracts or 1 to 5 µL of purified LSD1 enzyme to each sample well without inhibitor. Total volume should be 50 µL per well.
5. Sample Well With Inhibitor: Add 40 to 43 µL of LF2, 1 µL of LF3, 1 to 4 µL of your nuclear extracts or 1 to 4 µL of purified LSD1 enzyme, and 5 µL of inhibitor solution. Total volume should be 50 µL per well.
Note: (1) Follow the suggested well setup diagrams; (2) It is recommended to use 2 µg to 10 µg of nuclear extract per well or 10 ng to 100 ng of purified enzyme per well; (3) The concentration of inhibitors to be added into the sample wells can be varied (e.g., 1 µM to 1000 µM). However, the final concentration of the inhibitors before adding to the wells should be prepared with LF2 at a 1:10 ratio (e.g., add 0.5 µL of inhibitor to 4.5 µL of LF2), so that the original solvent of the inhibitor can be reduced to 1% of the reaction solution or less. The LSD1 inhibitor, Tranylcypromine (LF7) included in the kit can be used as a control inhibitor.
6. Tightly cover strip-well microplate with Adhesive Covering Film to avoid evaporation and incubate at 37°C for 60-120 min.
Note: (1) The incubation time may depend on intrinsic LSD1 activity. However, in general, 60-90 min incubation is suitable for active purified LSD1 enzymes and 90-120 min incubation is required for nuclear extracts; (2) The Adhesive Covering Film can be cut to the required size to cover the strips based on the number of strips to be used.
7. Remove the reaction solution from each well. Wash each well with 150 µL of the Diluted LF1 1X Wash Buffer each time for three times. Antibody Binding and Signal Enhancing
1. Add 50 µL of the Diluted LF5 to each well, then cover and incubate at room temperature for 60 min.
2. Remove the Diluted LF5 solution from each well.
3. Wash each well with 150 µL of the Diluted LF1 each time for three times.
4. Add 50 µL of the Diluted LF6 to each well, then cover and incubate at room temperature for 30 min.
5. Remove the Diluted LF6 solution from each well.

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6. Wash each well with 150 µL of the Diluted LF1 each time for four times.
Note: Ensure any residual wash buffer in the wells is removed as much as possible at each wash step.
Signal Detection
1. Add 50 µL of Fluorescence Development Solution to each well and incubate at room temperature for 2 to 4 min away from direct light. The Fluorescence Development Solution will turn pink in the presence of sufficient methylated products.
2. Read the fluorescence on a fluorescence microplate reader with 2 to 10 min at 530ex/590em 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.

Analysis

LSD1 Activity Calculation
Calculate the average duplicate readings for the sample wells and blank wells.
Calculate LSD1 activity or inhibition using the following formulas:
For simple calculation with a single standard control:
LSD1 Activity(RFU/min/mg) = (SampleRFU -BlankRFU)/(Protein Amount(ug)* x min**)*1000 □
* Protein amount (µg) added into the Enzymatic Reaction at step 4.
** Incubation time (minutes) at Enzymatic Reaction step 6.□ □ □
For accurate or specific activity calculation:
1. Generate a standard curve and plot RFU value versus amount of LF4 at each concentration point.
2. 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 formulas:
Demethylated Product(ng) = (SampleRFU -BlankRFU)/Slope
LSD1 Activity (ng/min/mg) = DemethylatedProduct(ng)/(Protein Amount(ug) x min*) x 1000
* Incubation time (minutes) at Enzymatic Reaction step 6.
For inhibition calculation:
Inhibitor 100% = [1-(InhibitorSampleRFU-BlankRFU)/(No InhibitorSampleRFU - BlankRFU)] * 100%