CREATIVE BIOMART®
Assay Kit

LSD1 Demethylase Activity/Inhibition Colorimetric Assay Kit

Product Information

Cat.No. Kit-0523

Product Overview

LSD1 Demethylase Activity/Inhibition Assay Kit (Colorimetric) is use for screening LSD1 demethylase inhibitors.

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 (Colorimetric) 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)

Storage

Upon receipt: (1) Store LD3, LD4, and LD6 at –20°C away from light; (2) Store LD1, LD5, LD7, LD8, and

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8-Well Assay Strips at 4°C away from light; (3) Store remaining components (LD2, LD9, 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 LD1 (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; and (2) check if a blue color is present in LD8 (Developer Solution), which would indicate contamination of the solution and should not be used. To avoid contamination, transfer the amount of LD8 required into a secondary container (tube or vial) before adding LD8 into the assay wells.

Kit Components

LD1 (10X Wash Buffer) 14 mLLD2 (LSD1 Assay Buffer) 4 mLLD3 (LSD1 Substrate, 50 μg/mL)* 60 μLLD4 (LSD1 Assay Standard, 50 μg/mL)* 10 μLLD5 (Capture Antibody, 1000 μg/mL*) 5 μLLD6 (Detection Antibody, 400 μg/mL)* 6 μLLD7 (LSD1 Inhibitor Tranylcypromine, 1 mM)* 20 μLLD8 (Developer Solution) 5 mLLD9 (Stop Solution) 5 mL8-Well Assay Strips (With Frame) 6 stripsAdhesive Covering Film 1 slice* Spin the solution down to the bottom prior to use.

Detection method Colorimetric

Compatible Sample Types

Cell Lysate

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 colorimetric 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 5 ng of purified LSD1 enzyme, which is about 20 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

Tel: 1-631-559-9269 1-516-512-3133
Email:info@creative-biomart.org
Fax:1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

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