CREATIVE BIOMART®
Assay Kit

JARID Demethylase Activity/Inhibition Colorimetric Assay Kit

Product Information

Cat.No.

Kit-0485

Product Overview

JARID Demethylase Activity/Inhibition Assay Kit (Colorimetric) is use for measuring activity or inhibition of total JARID.

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. The JARID family, except JARID2 (JARID1A, JARID1B, JARID1C and JARID1D), can remove tri-methylation from H3-K4. JARID demethylases are Jumonji-domain proteins and catalyze the removal of methylation by using a hydroxylation reaction with a requirement of iron and a-ketoglutarate as cofactors.

Applications

JARID Demethylase Activity/Inhibition Assay Kit (Colorimetric) is suitable for measuring activity or inhibition of total JARID usingnuclear extracts or subtype JARID (JARID1A-JARID1D) purified enzymes from a broad range of species such as mammalians, plant, fungal, and bacterial types, 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 JARIDs from recombinant proteins or isolated from cell/tissues.

Usage

For research use only (RUO)

Storage

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Upon receipt: (1) Store JC3, JC4, and JC6 at –20°C away from light; (2) Store JC1, JC5, JC7, Co-Factor 1, Co-Factor 2, Co-Factor 3, and 8-Well Assay Strips at 4°C away from light; (3) Store remaining components (JC2, JC8, 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 JC1 (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 JC7 (Developer Solution), which indicates contamination of the solution and should not be used. To avoid contamination, transfer the amount of JC7 required into a secondary container (tube or vial) before adding JC7 into the assay wells.

Kit Components

Component 48 Assays StorageJC1 (10X Wash Buffer) 14 ml 4°CJC2 (JARID Assay Buffer) 4 ml RTJC3 (JARID Substrate, 50 µg/ml)* 60 µl –20°CJC4 (JARID Assay Standard, 50 µg/ml)* 10 µl –20°CJC5 (Capture Antibody, 1000 µg/ml)* 5 µl 4°CJC6 (Detection Antibody, 400 µg/ml)* 6 µl –20°CJC7 (Developer Solution) 5 ml 4°CJC8 (Stop Solution) 5 ml RTCo-Factor 1* 30 µl 4°CCo-Factor 2* 30 µl 4°CCo-Factor 3* 30 µl 4°C8-Well Assay Strips (With Frame) 6 4°CAdhesive Covering Film 1 RTUser Guide 1 RT * Spin the solution down to the bottom prior to use.

Detection method Colorimetric

Features & Benefits

3 hour colorimetric procedure in a 96 stripwell microplate format allows for either manual or high throughput analysis.Directly measures JARID activity via a straightforward detection of JARID-converted demethylated products, rather than by-products, thus eliminating assay interference caused by thiol-containing chemicals such as DTT, GSH and 2-mercaptoethanol, or caused by detergents/ions such as tween-20, SDS, triton X-100, Fe, and Na.Both cell/tissue extracts and purified JARID proteins (including JARID1A, JARID1B, JARID1C, and JARID1D) can be used, which allows for the detection of inhibitory effects of JARID inhibitors in vivo and in vitro.Sensitivity is up to 1,000 times higher than formaldehyde release-based JARID assays, allowing activity to be colorimetrically detected from as low as 10 ng of purified JARID enzyme.Demethylated H3-K4 standard is included, allowing specific activity of JARID to be quantified.Accurate, reliable, and consistent with extremely low background signals.

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