CREATIVE BIOMART®
Assay Kit

JARID Demethylase Activity/Inhibition Fluorometric Assay Kit

Product Information

Cat.No.

Kit-0486

Product Overview

JARID Demethylase Activity/Inhibition Assay Kit (Fluorometric) is use for screening JARID 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. 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

The JARID Demethylase Activity/Inhibition Assay Kit (Fluorometric) inhibition of total JARID using nuclear extracts or subtype JARID (JARID1A through 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.

Usage

For research use only (RUO)

Storage

Upon receipt: (1) Store JD3, JD4, JD6, and JD7 at -20°C away from light; (2) Store JD1, JD5, JD8, Co-

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factor 1, Co-factor 2, Co-factor 3, and 8-Well Assay Strips at 4°C away from light; (3) Store remaining components (JD2, JD9, and Adhesive Covering Film) at room temperature.Note: (1) Check if JD1 (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.All components of the kit are stable for 6 months from the date of shipment, when stored properly.

Kit Components

JD1 (10X Wash Buffer) 14 mlJD2 (JARID Assay Buffer) 4 mlJD3 (JARID Substrate, 50 µg/ml)* 60 µlJD4 (JARID Assay Standard, 50 µg/ml)* 10 µlJD5 (Capture Antibody, 1000 µg/ml*) 5 µlJD6 (Detection Antibody, 400 µg/ml)* 6 µlJD7 (Fluoro-Developer)* 10 µlJD8 (Fluoro-Enhancer)* 10 µlJD9 (Fluoro-Dilution) 4 mlCo-factor 1* 30 µlCo-factor 2* 30 µlCo-factor 3* 30 µl8-Well Assay Strips (With Frame) 6Adhesive Covering Film 1* Spin the solution down to the bottom prior to use.

Detection method Fluorometric

Features & Benefits

• 3 hour fluorometric 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 2,000 times higher than formaldehyde release-based JARID assays, allowing activity to be fluorometrically detected from as low as 5 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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