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Data Sheet
JARID1C Homogeneous Assay Kit
Catalog # w60522

DESCRIPTION:
The *JARID1C Homogeneous Assay Kit* is designed to measure activity of the JARID1C for screening and profiling applications. JARID1C, also known as SMCX (Smcy homolog, X-linked), KDM5C, or XE169, is a JumonjiC (JmjC) and ARID domain-containing histone lysine demethylase that exhibits demethylation activity toward di- and trimethyl-lysine 4 (H3K4me2/3) on histone H3. The *JARID1C Homogeneous Assay Kit* comes in a convenient AlphaLISA® format, with biotinylated histone H3 peptide substrate, primary antibody, demethylase assay buffer, and purified JARID1C for 384 enzyme reactions. The key to the *JARID1C Homogeneous Assay Kit* is a highly specific antibody that recognizes demethylated substrate. With this kit, only three simple steps on a microtiter plate are required for demethylase activity detection. First, a sample containing JARID1C enzyme is incubated with the biotinylated substrate. Next, acceptor beads and primary antibody are added, then donor beads, followed by reading the Alpha-counts.

COMPONENTS:

Catalog #	Component	Amount	Storage	
w60123	JARID1C	20 µg	-80°C	***Avoid Freeze/ Thaw Cycles***
w62151M	Primary antibody 13	200 µl	-80°C	
	Biotinylated histone H3 peptide substrate	400 µl	-80°C	
	4x JARID1C assay buffer 1	2 ml	-20°C	
	4X JARID1C assay buffer 2 (Incomplete Buffer)	1 ml	-20°C	
	4x Detection buffer	2 ml	-20°C	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
AlphaLISA anti-mIgG acceptor beads, 5 mg/ml (PerkinElmer #AL105C)
AlphaScreen Streptavidin-conjugated donor beads, 5 mg/ml (PerkinElmer #6760002S)
Optiplate -384 (PerkinElmer #6007290)
AlphaScreen microplate reader
Adjustable micropipettor and sterile tips

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

CONTRAINDICATIONS: Green and blue dyes that absorb light in the AlphaScreen signal emission range (520-620 nm), such as Trypan Blue. Avoid the use of the potent singlet oxygen quenchers such as sodium azide (NaN_3) or metal ions (Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+} and Ni^{2+}). The presence of culture medium RPMI 1640 at >1% leads to signal reduction due to the presence of excess biotin and iron in this medium. MEM, which lacks these components, does not affect AlphaScreen assays.

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STABILITY: At least one year from date of receipt when stored as directed.

REFERENCE: Iwase S, Lan F, Bayliss P, *et al. Cell* 2007; **128**(6):1077-1088.

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

Step 1:

1) Prepare master mix: N wells × (2.5 µl 4× JARID1C assay buffer 1 + 1 µl Biotinylated substrate + 0.5 µl water).

2) Thaw JARID1C on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot JARID1C enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80℃ immediately. *Note: JARID1C is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

3) Dilute JARID1C in 1X JARID1C assay buffer 2 (Incomplete Buffer) at 16.7 ng/µl (50ng/3 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use. Note: The incomplete buffer, which does not contain α-ketoglutarate, provides a more accurate background value than a no-enzyme control.

4) Add 4 µl of master mixture to each well designated for the “Positive Control” and “Test Inhibitor”. For the "Blank", add 2.5 µl 4×JARID1C assay buffer 2 (Incomplete buffer) + 1 µl Biotinylated substrate + 0.5 µl water.

Reagent	Blank	Positive Control	Test Inhibitor
4x JARID1C assay buffer 1	–	2.5 µl	2.5 µl
4x JARID1C assay Buffer 2 (Incomplete buffer)	2.5 µl	–	–
Biotinylated Substrate	1 µl	1 µl	1 µl
Distilled water	0.5 µl	0.5 µl	0.5 µl
Test Inhibitor/Activator	–	–	3 µl
Inhibitor buffer (no inhibitor)	3 µl	3 µl	–
JARID1C (16.7 ng/µl)	3 µl	3 µl	3 µl
Total	10 µl	10 µl	10 µl

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5) Add 3 µl of inhibitor solution to each well designated “Test Inhibitor”. For the “Positive Control" and "Blank" add 3 µl of the same solution without inhibitor (Inhibitor buffer)
6) Initiate reaction by adding 3 µl of diluted JARID1C prepared as described above. Incubate at room temperature for one hour. *Note: All incubations are done with slow shaking on a rotator platform.*

Step 2:

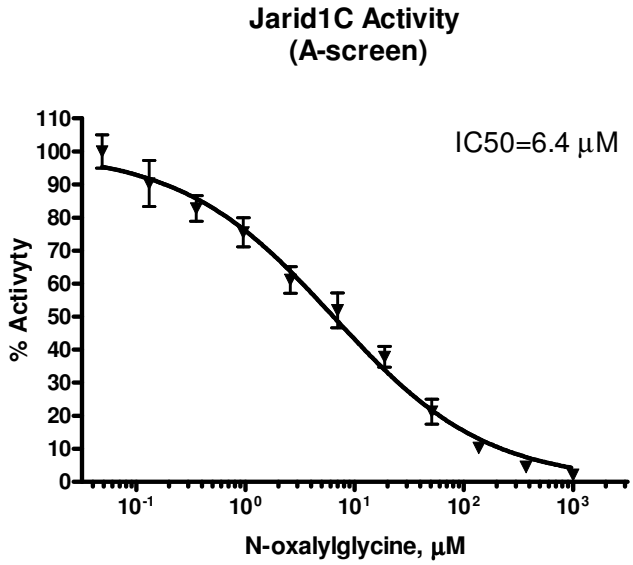
Note: Protect your samples from direct exposure to light!

1) Dilute anti-Mouse Acceptor beads (PerkinElmer #AL105C) 1:250-fold with 1x Detection buffer. Add 5 µl per well. Manually shake plate briefly.
2) Dilute “Primary antibody 13” 10-fold with 1x Detection buffer. Add 5 µl per well. Shake on a rotator platform for 30 minutes at room temperature.

Step 3:

1) Dilute Streptavidin-conjugated donor beads (PE #6760002S) 125-fold with 1x Detection buffer. Add 10 µl per well. Shake on a rotator platform for 15 minutes at room temperature.
2) Read Alpha-counts.

Example of Assay Results:



JARID1C enzyme activity, measured using the JARID1C Homogeneous Assay Kit, West Bioscience #w60522. *Data shown is lot-specific. For lot-specific information, please contact West Bioscience, Inc. at sale@westbioscience.com*

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