9891 Irvine Center Dr. Suite 200
Irvine, CA 92618
United States
Tel: 1.800.831.1518
Fax: 1.800.831.1518
Email: sale@westbioscience.com

Data Sheet
JARID1B Homogeneous Assay Kit
Catalog # w60523
Size: 384 reactions

DESCRIPTION:
The *JARID1B Homogeneous Assay Kit* is designed to measure activity of the JARID1B for screening and profiling applications. JARID1B, also known as PLU-1 and KDM5B, 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 *JARID1B Homogeneous Assay Kit* comes in a convenient AlphaLISA® format, with biotinylated histone H3 peptide substrate, primary antibody, demethylase assay buffer, and purified JARID1B for 384 enzyme reactions. The key to the *JARID1B 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 JARID1B 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	
w60132	JARID1B (KDM5A, RBBP2)	80 µg	-80℃	***Avoid Freeze/ Thaw Cycles***
w62151M	Primary antibody 13	200 µl	-80℃	
	Biotinylated histone H3 peptide substrate	500 rxns	-80℃	
	4x JARID1B assay buffer 1	2 ml	-80℃	
	4x JARID1B assay buffer 2 (Incomplete Buffer)	1 ml	-80℃	
w62312	4x Detection buffer 1	2 ml	-20℃	

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

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excess biotin and iron in this medium. MEM, which lacks these components, does not affect AlphaScreen® assays.

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

REFERENCE: Lahoud, M.H., *et al. Genome Res* **11** (8): 1327–34. .

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

Step 1:

1) Re-suspend lyophilized **Biotinylated histone H3 peptide substrate** in 500 µl of distilled water.

2) Prepare master mix: N wells × (2.5 µl **4x JARID1B assay buffer 1** + 1 µl **Biotinylated substrate** + 0.5 µl water).

3) Add 4 µl of master mixture to each well designated for the “Positive Control” and “Test Inhibitor”. For the "Blank", add 2.5 µl **4x JARID1B assay buffer 2** (Incomplete buffer) + 1 µl **Biotinylated substrate** + 0.5 µl water. *Note: The incomplete buffer, which does not contain α-ketoglutarate, provides a more accurate background value than a no-enzyme control.*

4) Thaw **JARID1B** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **JARID1B** enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: **JARID1B** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

5) Dilute **JARID1B** in **1x JARID1B assay buffer 2** (Incomplete Buffer) at 66.7 ng/µl (200 ng/3 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

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Reagent	Blank	Positive Control	Test Inhibitor
4x JARID1B assay buffer 1	–	2.5 µl	2.5 µl
4x JARID1B 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	–	–	3 µl
Inhibitor buffer (no inhibitor)	3 µl	3 µl	–
JARID1B (66.7 ng/µl)	3 µl	3 µl	3 µl
Total	10 µl	10 µl	10 µl

6) 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)

7) Initiate reaction by adding 3 µl of diluted **JARID1B** 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.

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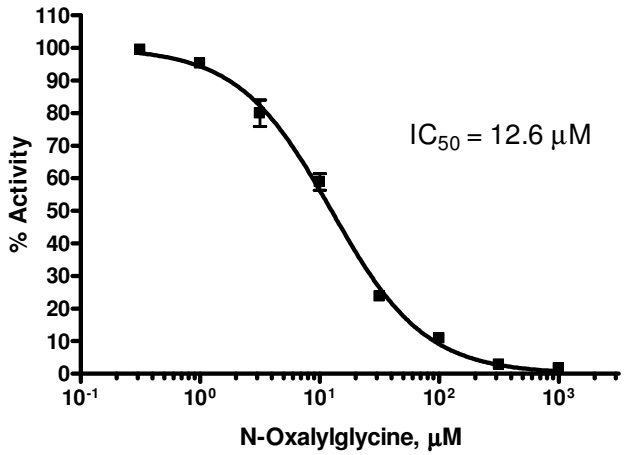
Example of Assay Results:



JARID1B enzyme activity, measured using the JARID1B Homogeneous Assay Kit, West Bioscience #w60523. *Data shown is lot-specific. For lot-specific information, please contact West Bioscience, Inc. at sale@westbioscience.com*