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Data Sheet
JARID1B Homogeneous Assay Kit
Catalog # 50512
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	
50121	JARID1B (KDM5A, RBBP2)	80 µg	-80°C	***Avoid Freeze/ Thaw Cycles***
52140M	Primary antibody 13	25 µl	-80°C	
	Biotinylated histone H3 peptide substrate (JARID)	500 rxns	-80°C	
52407	4x HDM Assay Buffer 2*	2 ml	-80°C	
52410	4x HDM Incomplete Assay Buffer	1 ml	-20°C	
52301	4x Detection buffer 1	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.

**This buffer was reformulated in May of 2015 with a different reducing agent to improve assay performance. The old formulation can still be purchased upon special request.*

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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.

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 HDM Assay Buffer 2** + 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 HDM Incomplete Assay 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 HDM Incomplete Assay Buffer** at 66.7 ng/µl (200 ng/3 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

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.*

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Reagent	Blank	Positive Control	Test Inhibitor
4x HDM Assay Buffer 2	—	2.5 µl	2.5 µl
4x HDM Incomplete Assay 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

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**" 100-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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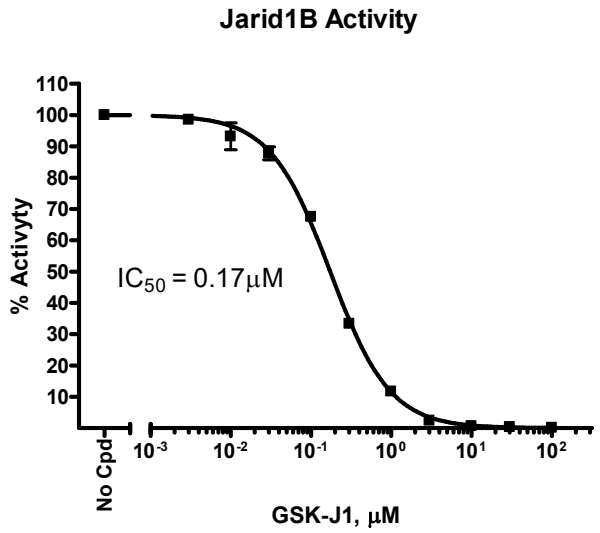
Example of Assay Results:



JARID1B enzyme activity, measured using the JARID1B Homogeneous Assay Kit, BPS Bioscience #50512. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog #	Size
JARID1A recombinant protein	50110	20 µg
JARID1B (PLU-1) recombinant protein	50121	20 µg
JARID1B (mouse) recombinant protein	50122	20 µg
JARID1C (SMCX) recombinant protein	50112	20 µg
JMJD1A recombinant protein	50130	20 µg
JMJD2A recombinant protein	50123	20 µg
JMJD2B recombinant protein	50104	20 µg
JMJD2C recombinant protein	50105	20 µg
JMJD2D recombinant protein	50117	20 µg
JARID1A Homogeneous Assay Kit	50510	384 reactions
JARID1C Homogeneous Assay Kit	50511	384 reactions
JMJD2A Homogeneous Assay Kit	50413	384 reactions
JMJD2B Homogeneous Assay Kit	50414	384 reactions
JMJD2C Homogeneous Assay Kit	50415	384 reactions

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