

**Data Sheet**  
***JMJD2B Homogeneous Assay Kit***  
**Catalog #k \* \$( &)**

**DESCRIPTION:** The *JMJD2B Homogeneous Assay Kit* is designed to measure JMJD2B activity for screening and profiling applications. JMJD2B is a JmjC-domain protein that exhibits demethylation activity toward H3-K9Me3 and H3-K36Me3. The *JMJD2B Homogeneous Assay Kit* comes in an AlphaLISA<sup>®</sup> format, with biotinylated histone H3 peptide substrate, primary antibody, demethylase assay buffer, and purified JMJD2B for 384 enzyme reactions. The key to the *JMJD2B 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 methyltransferase detection. First, a sample containing JMJD2B 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	
w62314	JMJD2B (KDM4B), GST-tag	60 µg	-80 °C	<b>Avoid freeze/thaw cycles!</b>
w62314	Primary antibody 5	40 µl	-80 °C	
	Biotinylated histone H3 peptide substrate (JMJD2B)	400 µl	-80 °C	
w62314	4x JMJD2B assay buffer	2 ml	-20 °C	
	4x JMJD2B assay buffer 2 (Incomplete buffer)	1 ml	-20 °C	
	4x Detection buffer	2 ml	-20 °C	

**MATERIALS REQUIRED BUT NOT SUPPLIED:**

AlphaLISA<sup>®</sup> anti-mIgG acceptor beads, 5 mg/ml (PerkinElmer #AL105C)  
 AlphaScreen<sup>®</sup> Streptavidin-conjugated donor beads, 5 mg/ml (PerkinElmer #6760002S)  
 Optiplate -384 (PerkinElmer #6007290)  
 AlphaScreen<sup>®</sup> 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<sub>3</sub>) or metal ions (Fe<sup>2+</sup>, Fe<sup>3+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup> and Ni<sup>2+</sup>). The presence of >1% RPMI 1640 culture medium leads to a signal reduction due to the presence of excess biotin and iron in this medium. MEM, which lacks these components, does not affect AlphaScreen assays.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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

**REFERENCE(S):**

1. Whetstine JR et al. *Cell* 2006; **125**: 467.
2. Fodor, B.D., *Genes & Dev.* 2006. **20**: 1557-1562.

**APPLICATION REFERENCE(S):**

Nakagawa-Yagi, Y., et al. *BMC Complement Altern Med.* 2012;**12(1)**:101.

**ASSAY PROTOCOL:**

All samples and controls should be tested in duplicate.

**Step 1:**

- 1) Thaw JMJD2B on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot JMJD2B enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: JMJD2B is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*
- 2) Dilute JMJD2B in 1x JMJD2B assay buffer or 1x Incomplete buffer at 30-50 ng/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.*
- 3) Using master mixes as much as possible, add the following reagents to the microwells, in duplicate:

	Positive Control	Test Sample	Blank
JMJD2B (30-50 ng/μl)	3 μl	3 μl	3 μl
4x JMJD2B assay buffer	2.5 μl	2.5 μl	–
4x Incomplete buffer	–	–	2.5 μl
Biotinylated substrate	1 μl	1 μl	1 μl
Test Inhibitor/Activator	–	X μl	–
H <sub>2</sub> O	3.5 μl	3.5 – X μl	3.5 μl
<b>Total</b>	<b>10 μl</b>	<b>10 μl</b>	<b>10 μl</b>

- 4) Add the entire reaction mixture (10 μl) to the well of a 384-well white plate. Incubate at room temperature for 1 hour.

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## Step 2:

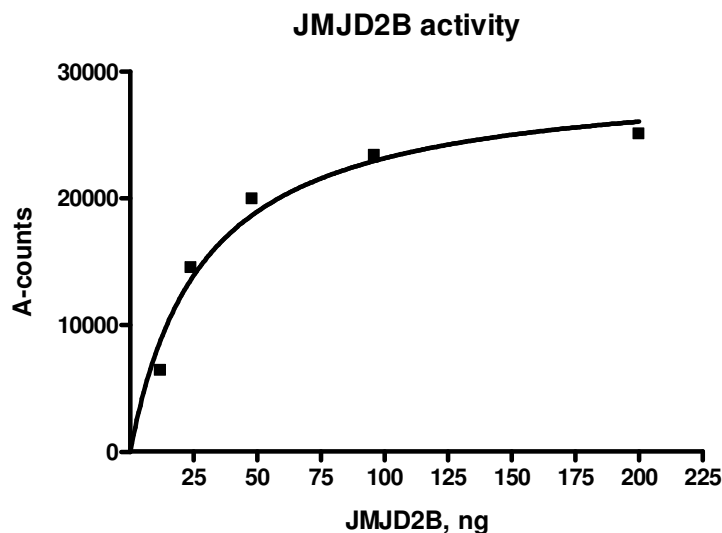
**Note: Protect your samples from direct exposure to light!**

- 1) Dilute anti-Mouse Acceptor beads (PerkinElmer #AL105C) 1:250-fold with 1x Detection buffer. Add 5  $\mu$ l per well. Shake plate briefly.
- 2) Dilute "Primary antibody 5" 50-fold with 1x Detection buffer. Add 5  $\mu$ l 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. Shake on a rotator platform for 15 minutes at room temperature.
- 2) Read Alpha-counts.

## Example of Assay Results:



JMJD2B enzyme activity, measured using the *JMJD2B Homogeneous Assay Kit*, West Bioscience Cat. #50414. *Data shown is lot-specific. For lot-specific information, please contact West Bioscience, Inc. at [sale@westbioscience.com](mailto:sale@westbioscience.com)*

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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](mailto:sale@westbioscience.com)

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