

## Data Sheet

### ***JMJD2E Homogeneous Assay Kit***

**Catalog # 50417**

**DESCRIPTION:** The *JMJD2E Homogeneous Assay Kit* is designed to measure activity of JMJD2E for screening and profiling applications. JMJD2E, also known as KDM4DL (lysine (K)-specific demethylase 4D-like) is a JmjC-domain protein that exhibits demethylation activity toward H3-K9Me3 and H3-K36Me3. The *JMJD2E Homogeneous Assay Kit* comes in a convenient AlphaLISA® format, with biotinylated histone H3 peptide substrate, primary antibody, demethylase assay buffer, and purified JMJD2E for 384 enzyme reactions. The key to the *JMJD2E 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 JMJD2E enzyme is incubated with the biotinylated substrate for two hours. Next, acceptor beads and primary antibody are added, then donor beads, followed by reading the Alpha-counts.

#### **COMPONENTS:**

Cat. #	Component	Amount	Storage	
50118	JMJD2E	40 µg	-80 °C	<b>(Avoid freeze/thaw cycles!)</b>
52140E	Primary antibody 5	40 µl	-80 °C	
	Biotinylated histone H3 peptide substrate	400 µl	-80 °C	
52305	4x JMJD2E assay buffer	2 ml	-20 °C	
	4x Detection buffer	2 ml	-20 °C	

#### **MATERIALS 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<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 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.

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**ASSAY PROTOCOL:**

All samples and controls should be tested in duplicate.

**Step 1:**

- 1) Thaw JMJD2E on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot JMJD2E enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: JMJD2E is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*
- 2) Dilute JMJD2E in 1X JMJD2E assay buffer at 40 ng/μl (100 ng/2.5 μl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.
- 3) Using master mixes as much as possible, add the following reagents to the microwells, in duplicate:

Reagent	Positive Control	Test Sample	Blank
JMJD2E (40 ng/μl)	2.5 μl	2.5 μl	–
4x JMJD2E assay buffer 1	2.5 μl	2.5 μl	2.5 μl
Biotinylated substrate	1 μl	1 μl	1 μl
Test Inhibitor/Activator	–	X μl	–
H <sub>2</sub> O	4 μl	4 – X μl	6.5 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.

**Step 2:**

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

- 1) Dilute anti-Mouse Acceptor beads (PerkinElmer #AL105C) 1:250 with 1x Detection buffer. Add 5 μl per well. Shake plate briefly.
- 2) Dilute "Primary antibody 5" 50-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.

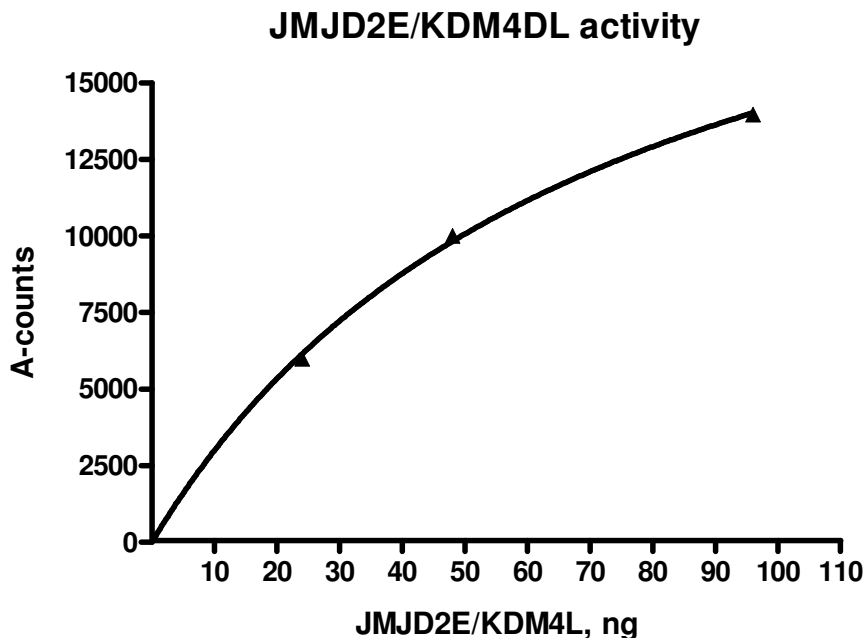
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**Example of Assay Results:**



JMJD2E enzyme activity, measured using the JMJD2E Homogeneous Assay Kit, BPS Bioscience #50417. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at [info@bpsbioscience.com](mailto:info@bpsbioscience.com)*

**REFERENCE:** Whetstine, J.R. et al., *Cell* 2006; **125**: 467.

**RELATED PRODUCTS:**

JMJD2A recombinant protein	#50103	20 µg
JMJD2B recombinant protein	#50104	20 µg
JMJD2C recombinant protein	#50105	20 µg
JMJD2E (KDM4DL) recombinant protein	#50118	20 µg
JMJD3 recombinant protein	#50115	20 µg
JMJD6 recombinant protein	#50140	20 µg
JMJD2A Homogeneous Assay Kit	#50413	384 rxns
JMJD2B Homogeneous Assay Kit	#50414	384 rxns
JMJD2C Homogeneous Assay Kit	#50415	384 rxns

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