6044 Cornerstone Court West, Suite E
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet
JMJD3 Homogeneous Assay Kit
Catalog #50416

DESCRIPTION:
The *JMJD3 Homogeneous Assay Kit* is designed to measure activity of the JMJD3 for screening and profiling applications. JMJD3 is a JmjC-domain protein that exhibits demethylation activity toward di- and trimethyl-lysine 27 (H3K27me2/3) on histone H3. The *JMJD3 Homogeneous Assay Kit* comes in a convenient AlphaLISA® format, with biotinylated histone H3 peptide substrate, primary antibody, demethylase assay buffer, and purified JMJD3 for 384 enzyme reactions. The key to the *JMJD3 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 JMJD3 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:

Component	Amount	Storage	
JMJD3	40 µg	-80°C	***Avoid Freeze/ Thaw Cycles***
Primary antibody 6	40 µl	-80°C	
Biotinylated histone H3 peptide substrate	400 µl	-80°C	
4x JMJD3 assay buffer 1	2 ml	-20°C	
4X JMJD3 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-rIgG acceptor beads, 5 mg/ml (PerkinElmer #AL104C)
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.

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

6044 Cornerstone Court West, Suite E
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

REFERENCE: Swigut T., Wysocka J. *Cell* 2007; **131**(1): 29-32.

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

Step 1:

1) Prepare master mix: N wells × (2.5 µl 4×JMJD3 assay buffer 1 + 1 µl Biotinylated substrate + 0.5 µl water).

2) Thaw JMJD3 on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot JMJD3 enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: JMJD3 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

3) Dilute JMJD3 in 1X JMJD3 assay buffer 2 (Incomplete Buffer) at 25-33 ng/µl (75-99 ng/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×JMJD3 assay buffer 2 (Incomplete buffer) + 1 µl Biotinylated substrate + 0.5 µl water.

Reagent	Blank	Positive Control	Test Inhibitor
4x JMJD3 assay buffer 1	—	2.5 µl	2.5 µl
4x JMJ D3 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	–
JMJD3 (25-33 ng/µl)	3 µl	3 µl	3 µl
Total	10 µl	10 µl	10 µl

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)

6044 Cornerstone Court West, Suite E
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

6) Initiate reaction by adding 3 µl of diluted JMJD3 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!

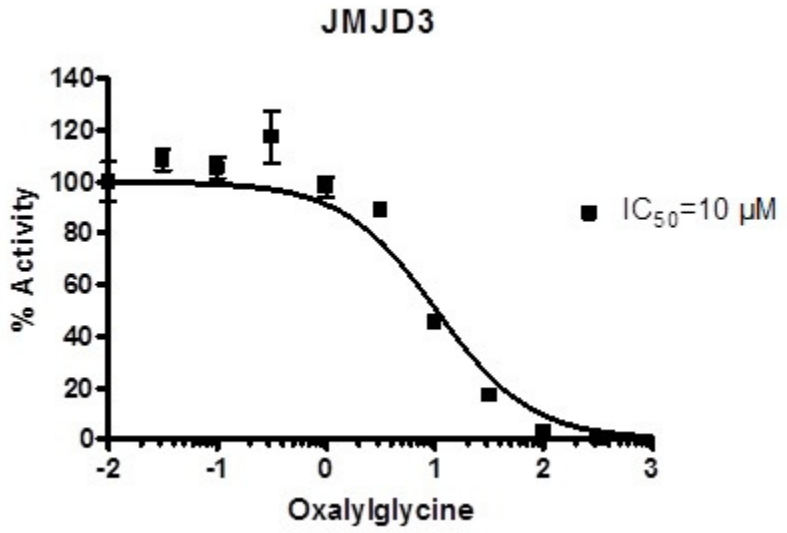
1) Dilute anti-Rabbit Acceptor beads (PerkinElmer #AL104C) 1:250-fold with 1x Detection buffer. Add 5 µl per well. Manually shake plate briefly.
2) Dilute "Primary antibody 6" 50-fold with 1x Detection buffer. Add 5 µl per well. Shake on a rotator platform for 30 minutes at room temperature.

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.

6044 Cornerstone Court West, Suite E
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Example of Assay Results:



JMJD3 enzyme activity, measured using the JMJD3 Homogeneous Assay Kit, BPS Bioscience #50416. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

JMJD3 (KDM6B) recombinant protein	#50115	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
JMJD2E recombinant protein	#50118	20 µg
JMJD2A Homogeneous Assay Kit	#50413	384 reactions
JMJD2B Homogeneous Assay Kit	#50414	384 reactions
JMJD2C Homogeneous Assay Kit	#50415	384 reactions
JMJD2E Homogeneous Assay Kit	#50417	384 reactions
JMJD2C Assay Kit, Chemiluminescence	#50405	96 reactions
4x JMJD2C Assay Buffer	#52304	10 ml