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Data Sheet

JMJD2C Homogeneous Assay Kit

Catalog #50415

DESCRIPTION: The *JMJD2C Homogeneous Assay Kit* is designed to measure JMJD2C activity for screening and profiling applications. JMJD2C is a JmjC-domain protein that exhibits demethylation activity toward H3-K9Me3 and H3-K36Me3. The *JMJD2C Homogeneous Assay Kit* comes in a convenient AlphaLISA® format, with biotinylated histone H3 peptide substrate, primary antibody, demethylase assay buffer, and purified JMJD2C for 384 enzyme reactions. The key to the *JMJD2C 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 JMJD2C 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	
50105	JMJD2C (GASC1, KDM4C)	60 µg	-80°C	***(Avoid freeze/thaw cycles!)***
52140E	Primary antibody 5	40 µl	-80°C	
	Biotinylated histone H3 peptide substrate	400 µl	-80°C	
52304	4x JMJD2C assay buffer	2 ml	-20°C	
	4x JMJ assay buffer 2 (Incomplete buffer)	1 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_3) or metal ions ($Fe2^+$, $Fe3^+$, $Cu2^+$, $Zn2^+$ and $Ni2^+$). 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.

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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.* **12(1):**101 (2012).

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

Step 1:

1) Prepare master mix: N wells × (2.5 µl 4×JMJD2C assay buffer + 1 µl Biotinylated substrate + 1 µl water).

2) Thaw JMJD2C on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot JMJD2C enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: JMJD2C is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

3) Dilute JMJD2C in 1X JMJD2C assay buffer at 30-50 ng/µl (90-150 ng/3 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

4) Add 4.5 µl of master mixture to each well designated for the "Positive Control" and "Test Inhibitor". For the "Blank", add 2.5 µl 4×JMJ assay buffer 2 (Incomplete buffer) + 1 µl Biotinylated substrate + 1 µl water. Note: The incomplete buffer, which does not contain α-ketoglutarate, provides a more accurate background value than a no-enzyme control.

Reagent	Blank	Positive Control	Test Inhibitor
4x JMJD2C assay buffer	–	2.5 µl	2.5 µl
4x JMJ assay buffer 2 (Incomplete buffer)	2.5 µl	–	–
Biotinylated Substrate	1 µl	1 µl	1 µl
H2O	1 µl	1 µl	1 µl
Test Inhibitor/Activator	–	–	2.5 µl
Inhibitor buffer (no inhibitor)	2.5 µl	2.5 µl	–
JMJD2C (30-50 ng/µl)	3 µl	3 µl	3 µl
Total	10 µl	10 µl	10 µl

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5) Add 2.5 µl of inhibitor solution of each well designated “Test Inhibitor”. For the “Positive Control" and "Blank" add 2.5 µl of the same solution without inhibitor (Inhibitor buffer).
6) Initiate reaction by adding 3 µl of diluted JMJD2C prepared as described above. Incubate at room temperature for one hour.

Step 2:

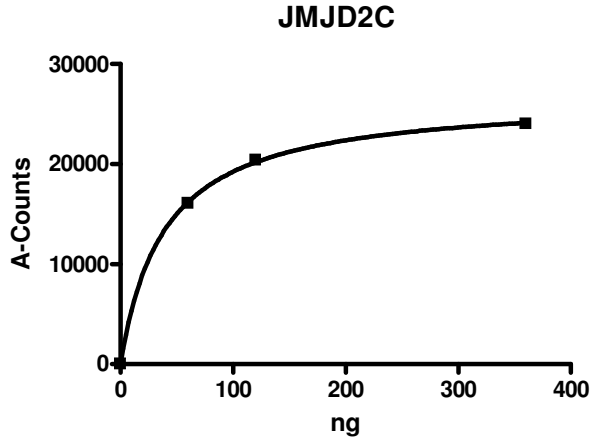
Note: Protect your samples from direct exposure to light!

1) Dilute anti-Mouse Acceptor beads (PerkinElmer #AL105C) 1:250-fold with 1x Detection buffer. Add 5 µl per well. 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.

Example of Assay Results:



JMJD2C enzyme activity, measured using the *JMJD2C Homogeneous Assay Kit*, BPS Bioscience Cat. #50415. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

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RELATED PRODUCTS:

Product Name	Catalog #	Size
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
JMJD2E Homogeneous Assay Kit	50417	384 reactions
JMJD2C Assay Kit, Chemiluminescence	50405	96 reactions
4x JMJD2C Assay Buffer	52304	10 ml

To place your order, please contact us by Phone **1.858.829.3082**, Fax **1.858.481.8694**
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