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Data Sheet
JMJD2D Chemiluminescent Assay Kit
Catalog #50418

DESCRIPTION: The *JMJD2D Chemiluminescent Kit* is designed to measure activity of the JMJD2D for screening and profiling applications. JMJD2D is a JmjC-domain protein that exhibits demethylation activity toward H3-K9Me3 and H3-K9Me2. The *JMJD2D Chemiluminescent Assay Kit* comes in a convenient format, with 8-well strips pre-coated with the methylated histone H3 peptide substrate, primary antibody, the secondary HRP-labeled antibody, demethylase assay buffer, and purified JMJD2D for 100 enzyme reactions. The key to *the JMJD2D Chemiluminescent 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 JMJD2D enzyme is incubated with a sample containing assay buffer for one hour. Next, primary antibody is added. Finally, the plate is treated with an HRP-labeled secondary antibody followed by addition of the HRP substrate to produce chemiluminescence that can then be measured using a chemiluminescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
50117	JMJD2D	3 x 14 µg	-80°C	***Avoid Freeze/ Thaw Cycles***
52140E	Primary antibody 5	12.5 µl	-80°C	
	Secondary HRP-labeled antibody 1	10 µl	-80°C	
	4x JMJD2D assay buffer	3 ml	-20°C	
	Blocking buffer	50 ml	+4°C	
	HRP chemiluminescent substrate A	6 ml	+4°C	
	HRP chemiluminescent substrate B	6 ml	+4°C	
	8-well strip plate module, precoated with histone substrate	1 (12 x 8-well strips)	+4°C	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
TBST buffer (1 x TBS, pH 8.0, containing 0.05% Tween20)
Luminometer or fluorescent microplate reader capable of reading chemiluminescence
Adjustable micropipettor and sterile tips
Rotating or rocker platform

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

CONTRAINDICATIONS: DMSO >1%, strong acids or bases, ionic detergents, high salt

"*Note: *The buffer in this kit 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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STABILITY: At least one year from date of receipt when stored as directed.

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

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

Step 1:

1) Rehydrate the microwells by adding 200 µl of TBST buffer (1x TBS, pH 8.0, containing 0.05% Tween-20) to every well. Incubate 15 minutes at room temperature. Tap the plate onto clean paper towels to remove liquid.

2) Prepare master mix: N wells × (7.5 µl **4x JMJD2D Assay Buffer** + 17.5 µl distilled water). Add 25 µl of master mixture to each well.

3) Add 5 µl of inhibitor solution to each well designated "Test Inhibitor". For the "Positive Control" and "Blank" add 5 µl of the same solution without inhibitor (Inhibitor buffer). *Note: Keep final DMSO concentration ≤1%.*

	Blank	Positive Control	Test Inhibitor
4x JMJD2D assay buffer	7.5 µl	7.5 µl	7.5 µl
Distilled water	17.5 µl	17.5 µl	17.5 µl
Test Inhibitor/Activator	–	–	5 µl
Inhibitor buffer (no inhibitor)	5 µl	5 µl	–
1x JMJD2D assay buffer	20 µl	–	–
JMJD2D (20 ng/µl)	–	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

4) Dilute 1 part **4x JMJD2D assay buffer** with 3 parts distilled water (4-fold dilution) to make **1x JMJD2D assay buffer.** Make only a sufficient quantity needed for the assay; store remaining stock solution in aliquots at -20°C.

5) Add 20 µl of **1x JMJD2D buffer** to wells designated as "Blank".

6) Thaw **JMJD2D** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **JMJD2D** enzyme into single use aliquots. Store remaining

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undiluted enzyme in aliquots at -80°C. *Note: **JMJD2D** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

7) Dilute **JMJD2D** in **1x JMJD2D assay buffer** at 20 ng/µl. Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

8) Initiate reaction by adding 20 µl of **diluted JMJD2D** prepared as described above to wells designated "Positive Control" and "Test Inhibitor". Incubate at room temperature for one hour.

9) Wash the plate three times with TBST buffer. Blot dry onto clean paper towels.

10) Add 100 µl of **Blocking buffer** to every well. Shake on a rotating platform for 10 min. Remove the supernatant from the wells.

Step 2:

1) Dilute "**Primary antibody 5**" 800-fold with **Blocking buffer**.

2) Add 100 µl per well. Incubate 1 hour at room temperature with slow shaking.

3) Wash strip plate with TBST buffer and incubate in **Blocking buffer** as described in steps 1-9 and 1-10.

Step 3:

1) Dilute "**Secondary HRP-labeled antibody 1**" 1,000-fold with **Blocking buffer**.

2) Add 100 µl per well. Incubate for 30 min. at room temperature with slow shaking.

3) Wash strip plate with TBST buffer and incubate in **Blocking buffer** as described in steps 1-9 and 1-10.

4) Just before use, mix on ice 50 µl **HRP chemiluminescent substrate A** and 50 µl **HRP chemiluminescent substrate B** and add 100 µl per well. Discard any unused chemiluminescent reagent after use.

5) Immediately read sample in a luminometer or microtiter-plate reader capable of reading chemiluminescence.

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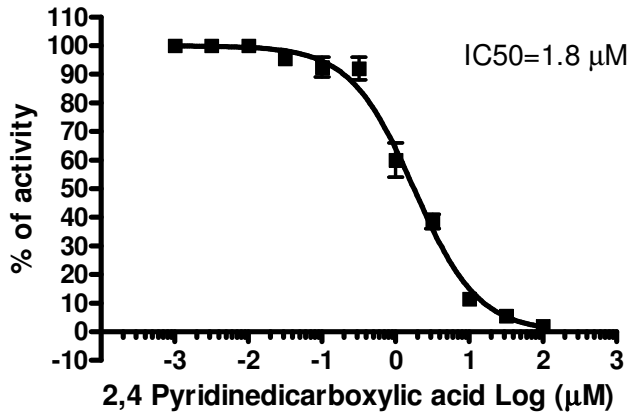
Example of Assay Results:



JMJD2D enzyme activity, measured using the *JMJD2D Chemiluminescent Kit*, BPS Bioscience #50418. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog #	Size
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

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is same as "blank" value.	JMJD2D has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh JMJD2D, BPS Bioscience #50117. Store enzyme in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Antibody reaction is insufficient	Increase time for primary antibody incubation. Avoid freeze/thaw cycles of antibodies.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity of light detection.
	Chemiluminescent reagents mixed too soon	Chemiluminescent solution should be used within 15 minutes of mixing. Ensure both reagents are properly mixed.
Luminescent signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
Background (signal to noise ratio) is high	Insufficient washes	Increase number of washes. Increase wash volume. Increase Tween-20 concentration to 0.1% in TBST.
	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of JMJD2D, BPS Bioscience #50117 to create a standard curve.

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