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Data Sheet

JMJD3 Chemiluminescent Assay Kit

Catalog # 50406
Size: 96 reactions

DESCRIPTION: The *JMJD3 Chemiluminescent Assay Kit* is designed to measure JMJD3 activity 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 Chemiluminescent Assay Kit* comes in a convenient format, with 8-well strips precoated with the methylated histone H3 peptide substrate, primary antibody, the secondary HRP-labeled antibody, demethylase assay buffer, and purified JMJD3 for 100 enzyme reactions. The key to the *JMJD3 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 JMJD3 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	
50115	JMJD3 (KDM6B)	4 µg	-80°C	***Avoid freeze/ thaw cycles!***
52140F	Primary antibody 6	12.5 µl	-80°C	
52131H	Secondary HRP-labeled antibody 2	10 µl	-80°C	
52306	4x JMJD3 direct assay buffer	3 x 1 ml	-80°C	
52100	Blocking buffer 4	50 ml	+4°C	
	HRP chemiluminescent substrate A (transparent bottle)	6 ml	+4°C	
	HRP chemiluminescent substrate B (brown bottle)	6ml	+4°C	
	8-well strip plate module pre-coated with histone substrate	1	+4°C	

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

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

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

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STABILITY: One year from date of receipt when stored as directed.

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

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 strip plate onto clean paper towels to remove liquid.

2) Prepare master mix: N wells × (7.5 µl **4x JMJD3 Direct Assay Buffer** + 12.5 µl water). Add 20 µl of master mixture to each well.

3) Add 10 µl of inhibitor solution of each well designated "Test Inhibitor." For the "Positive Control" and "Blank," add 10 µl of the same solution without inhibitor (Inhibitor buffer). *Note: Keep final DMSO concentration ≤1%.*

	Blank	Positive Control	Test Inhibitor
4x JMJD3 Direct Assay Buffer	7.5 µl	7.5 µl	7.5 µl
Distilled water	12.5 µl	12.5 µl	12.5 µl
Test Inhibitor/Activator	-	-	10 µl
Inhibitor buffer (no inhibitor)	10 µl	10 µl	-
1x JMJD3 Direct Assay Buffer	20 µl	-	-
JMJD3 (KDM6B) (2 ng/µl)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

4) Dilute 1 part **4x JMJD3 Direct Assay Buffer** with 3 parts distilled water (4-fold dilution) to make **1x JMJD3 Direct Assay Buffer**. Make only sufficient quantity needed for the assay; store remaining stock solution in aliquots at -20°C.

5) Add 20 µl of **1x JMJD3 Direct Assay Buffer** to wells designated as "Blank."

6) Thaw **JMJD3 (KDM6B)** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **JMJD3 (KDM6B)** enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: **JMJD3** **(KDM6B)** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

7) Dilute **JMJD3 (KDM6B)** in **1x JMJD3 Direct Assay Buffer** at 2 ng/µl (40 ng/reaction). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

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8) Initiate reaction by adding 20 µl of diluted **JMJD3 (KDM6B)** prepared as described above to wells designated "Positive Control" and "Test Inhibitor." Incubate at room temperature for one hour.

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

10) Add 100 µl of **Blocking buffer 4** to every well. Shake on a rotating platform for 10 minutes. Remove the supernatant from the wells.

Step 2:

1) Dilute "**Primary antibody 6"** 800-fold with **Blocking buffer 4**.

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 4** as described in steps 1-9 and 1-10.

Step 3:

1) Dilute "**Secondary HRP-labeled antibody 2"** 1,000-fold with **Blocking buffer 4**.

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 4** as described in steps 1-9 and 1-10.

4) Just before use, mix on ice 50 µl of **HRP chemiluminescent substrate A** and 50 µl of **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.

Reading Chemiluminescence:

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

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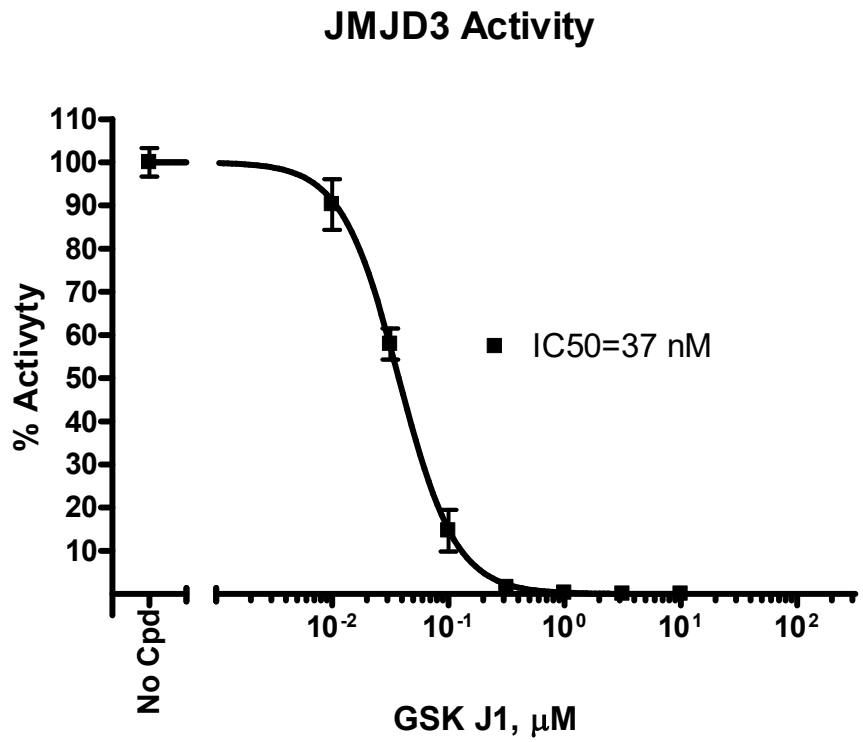
To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second. Delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

Example of Assay Results:



JMJD3 (KDM6B) enzyme activity, measured using the JMJD3 Chemiluminescent Assay Kit, BPS Bioscience #50406. *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	Catalog #	Size
JMJD3 recombinant protein	50115	20 µg
JMJD1A recombinant protein	50130	20 µg
JMJD2A recombinant protein	50123	100 µg
JMJD2B recombinant protein	50104	100 µg
JMJD2C recombinant protein	50105	100 µg
JMJD2D recombinant protein	50117	100 µg
JMJD2E recombinant protein	50118	100 µg
JMJD2A Homogeneous Assay Kit	50413	384 rxns
JMJD2B Homogeneous Assay Kit	50414	384 rxns
JMJD2C Homogeneous Assay Kit	50415	384 rxns
JMJD2C Chemiluminescent Assay Kit	50405	96 rxns
JMJD2E Homogeneous Assay Kit	50417	384 rxns
LSD1 Fluorescent Assay Kit (96 well)	50106	96 rxns
LSD1 recombinant protein	50100	50 µg
JARID1A recombinant protein	50110	20 µg
JARID1B recombinant protein	50121	20 µg
LSD1 recombinant protein	50100	50 µg

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is same as "blank" value.	JMJD3 has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh JMJD3, BPS Bioscience #50115. 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. See section on "Reading Chemiluminescence" above.
	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	Be sure to include blocking steps after wash steps. 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 JMJD3, BPS Bioscience #50115 to create a standard curve.