JMJD2D (KDM4D) Chemiluminescent Assay Kit

Description
The JMJD2D (KDM4D) Chemiluminescent Assay Kit is an ELISA (enzyme-linked immunosorbent assay)-based assay designed to measure the demethylase activity of JMJD2D (Jumonji domain-containing protein 2D) for screening and profiling applications. The JMJD2D (KDM4D) assay kit comes in a convenient 96-well format, with enough purified recombinant JMJD2D enzyme (amino acids 2-354), pre-coated plate with histone substrate, all the reagents necessary for assay detection and blocking buffer for 96 enzyme reactions.

Background
JMJD2D (Jumonji domain -containing protein 2D), also known as KDM4D, belongs to the JMJD2 subfamily of JMJD histone lysine demethylase proteins. It is involved in demethylating H3K9me2/3 and H1.4K26, participating in DNA replication and repair and transcription. It acts as an epigenetic regulator in androgen-receptor activation, inflammation, and tumor progression. JMJD2D has been linked to liver, colorectal, and pancreatic cancer. JMJD2D acts on the β-catenin pathway, by demethylating β-catenin target gene promoters and bringing β-catenin in proximity to them. Amongst other roles, it also participates in promoting glycolysis in cancer cells, likely by acting on the HIF1α (hypoxia inducible factor 1 alpha) pathway. The use of JMJD2D inhibitors is an area of interest that requires further development, and the development of active inhibitors will be beneficial in cancer therapy.

Applications
Study enzyme kinetics and screen small molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
50117	JMJD2D (KDM4D), GST-Tag*	40 µg	-80°C
52140E	Primary Antibody 5	12.5 µl	-80°C
52130H	Secondary HRP-Labeled Antibody 1	10 µl	-80°C
	4x JMJD2D Direct Assay Buffer	3 x 1 ml	-80°C
52100	Blocking Buffer 4	50 ml	+4°C
	HRP Substrate A (translucent bottle)	6 ml	Room Temp
	HRP Substrate B (brown bottle)	6 ml	Room Temp
	White plate pre-coated with Histone-Substrate	1 plate	Room Temp

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- TBST Buffer (1x Tris-buffered saline, pH 8, containing 0.05% Tween-20)
- Luminometer or microplate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

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Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

The JMJD2D (KDM4D) Chemiluminescent Assay Kit is compatible with up to 1% final DMSO concentration. Avoid the use of strong acids and bases, ionic detergents and high salt concentrations.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include "Blank", "Positive Control" and "Test Inhibitor" conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using 2,4-Pyridine Dicarboxylic Acid as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

Step 1

1. Rehydrate each pre-coated well with 200 µl of TBST Buffer.

2. Incubate 15 minutes at Room Temperature (RT).

3. Tap the plate onto clean paper towel to remove the liquid.

4. Wash the plate three times using 200 µl TBST Buffer per well.

5. Tap the plate onto clean paper towel to remove the liquid.

6. Prepare a Master Mix (25 µl/well): N x wells (7.5 µl of 4x JMJD2D Direct Assay Buffer + 17.5 µl of distilled water).

7. Add 25 µl of Master Mix to each well.

8. Prepare the Test Inhibitor (5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   8.1 If the Test Inhibitor is soluble in water, prepare a solution of the compound that is 10-fold higher than the final desired concentration in TBST.

   For the positive and negative controls, use TBST Buffer (Diluent Solution).

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OR

8.2 If the Test Inhibitor is dissolved in DMSO, prepare a solution of the compound in 100% DMSO that is 100-fold higher than the highest concentration of the serial dilution. Then dilute 10-fold with TBST Buffer (at this step the compound concentration is 10-fold higher than the desired final concentration). The concentration of DMSO in the dilution is now 10%.

Prepare serial dilutions of the Test Inhibitor at concentrations 10-fold higher than the desired final concentrations using 10% DMSO in TBST Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in TBST Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

9. Add 5 µl of inhibitor solution to the "Test Inhibitor" wells.

10. Add 5 µl of Diluent Solution to the "Blank" and "Positive Control" wells.

11. Dilute 4-fold the 4x JMJD2D Direct Assay Buffer with distilled water. This makes 1x JMJD2D Direct Assay Buffer.

12. Thaw **JMJD2D** on ice. Briefly spin the tube containing the enzyme to recover its full content.

13. Dilute JMJD2D to 20 ng/µl in 1x JMJD2D Direct Assay Buffer (20 µl/well).

14. Tap the plate onto clean paper towel to remove the liquid.

15. Add 20 µl of 1x JMJD2D Direct Assay Buffer to the "Blank" wells.

16. Add 20 µl of diluted JMJD2D solution to the "Positive Control" and "Test Inhibitor" wells.

17. Incubate at RT for 1 hour.

	Blank	**Positive Control**	**Test Inhibitor**
Master Mix	25 µl	25 µl	25 µl
Test Inhibitor	-	-	15 µl
Diluent Solution	5 µl	5 µl	-
Diluted JMJD2D (20 ng/µl)	-	20 µl	20 µl
1x JMJD2D Direct Assay Buffer Diluent Solution	20 µl	-	-
Total	**50 µl**	**50 µl**	**50 µl**

18. Wash the plate three times using 200 µl TBST Buffer per well.

19. Tap the plate onto clean paper towel to remove the liquid.

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20. Add 100 µl of Blocking Buffer 4 into each well.
21. Incubate at RT for 10 minutes.
22. Tap the plate onto clean paper towel to remove the Blocking Solution.
23. Wash the plate three times using 200 µl TBST Buffer per well.
24. Tap the plate onto clean paper towel to remove the liquid.

Step 2:

1. Dilute 800-fold the Primary Antibody 5 with Blocking Buffer 4 (100 µl/well).
2. Add 100 µl of diluted Primary Antibody 5 to each well.
3. Incubate 1 hour at RT with gentle agitation.
4. Wash the plate three times using 200 µl TBST Buffer per well.
5. Add 100 µl of Blocking Buffer 4 into each well.
6. Incubate at RT for 10 minutes.
7. Tap the plate onto clean paper towel to remove the Blocking Solution.
8. Wash the plate three times using 200 µl TBST Buffer per well.
9. Tap the plate onto clean paper towel to remove the liquid.

Step 3:

1. Dilute 1000-fold the Secondary HRP-Labeled Antibody 1 with Blocking Buffer 4 (100 µl/well).
2. Add 100 µl of diluted Secondary Antibody 1 to each well.
3. Incubate 30 minutes at RT with gentle agitation.
4. Wash the plate three times using 200 µl TBST Buffer per well.
5. Tap the plate onto clean paper towel to remove the liquid.
6. Add 100 µl of Blocking Buffer 4 into each well.
7. Incubate at RT for 10 minutes.

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8. Tap the plate onto clean paper towel to remove the Blocking Solution.

9. Wash the plate three times using 200 µl TBST Buffer per well.

10. Tap the plate onto clean paper towel to remove the liquid.

Step 4:

1. Just before use, mix 1 volume of HRP Substrate A and 1 volume of HRP Substrate B (100 µl of mix/well).

2. Add 100 µl of mix per well.

3. Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence.

4. The "Blank" value should be subtracted from all other values.

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. 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 Results

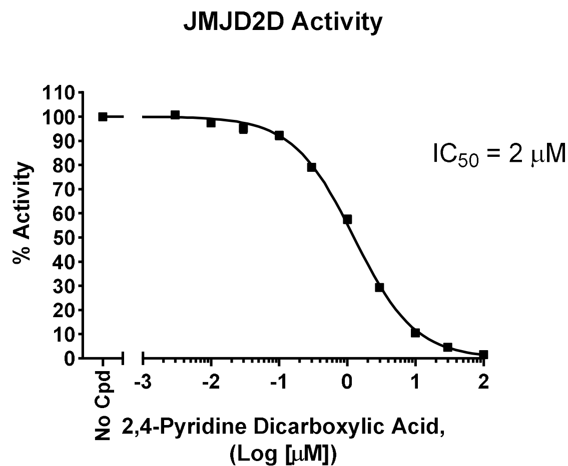

Figure 2: Inhibition of JMJD2D activity by 2, 4-Pyridine Dicarboxylic Acid.

JMJD2D activity was measured in the presence of increasing concentrations of 2, 4-Pyridine Dicarboxylic Acid.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

References

Manni W., *et al.*, 2022 *Signal Transduct Target Ther* 7:304.
Chen Q., *et al.*, 2022 *Cancers (Basel)* 14(12):2841.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
JMJD2B (JMJD2B) Homogeneous Assay Kit	50414	384 reactions
JMJD2C (KDM4C) Homogeneous Assay Kit	50415	384 reactions
JMJD2D (JMJD2D) Homogeneous Assay Kit	79838	384 reactions
JMJD2E (KDM4DL) Homogeneous Assay Kit	50417	384 reactions
Anti-JMJD2D (KDM4D) polyclonal antibody	25300	100 µl

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