Description

The JMJD2C (KDM4C) Chemiluminescent Assay Kit is an ELISA (enzyme-linked immunosorbent assay)-based assay designed to measure the demethylase activity of JMJD2C (Jumonji domain-containing protein 2C) for screening and profiling applications. The JMJD2C (KDM4C) assay kit comes in a convenient 96-well format, with enough purified recombinant JMJD2C enzyme (amino acids 2-372), pre-coated plate with histone substrate, all the reagents necessary for assay detection and blocking buffer for 96 enzyme reactions.

Background

JMJD2C (Jumonji domain -containing protein 2C), also known as KDM4C, belongs to the JMJD2 subfamily of JMJD histone lysine demethylase proteins. It is involved in demethylating H3K9me2/3, participating in epigenetic regulation. JMJD2C has been linked to colorectal cancer and uveal melanoma, where it also participates in cisplatin treatment resistance. JMJD2C promotes expression of MDM2 (mouse double minute-2 homolog) by demethylating its promoter. MDM2 in turn promotes the ubiquitination and degradation of p53 and increased levels of IL15Ra (interleukin 15 receptor subunit alpha), a pathway that supports cells to resist cisplatin treatment and uveal melanoma cell growth. The use of JMJD2C inhibitors is an area of interest that requires further development, and the development of active inhibitors will be beneficial in the cancer therapy field.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
50105	JMJD2C, GST-Tag*	20 µg	-80°C
52140E	Primary Antibody 5	12.5 µl	-80°C
52130H	Secondary HRP-Labeled Antibody 1	10 µl	-80°C
	4x JMJD2C 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

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 JMJD2C (KDM4C) 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).

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 (20 µl/well): N x wells (7.5 µl of 4x JMJD2C Direct Assay Buffer + 12.5 µl of distilled water).

7. Add 20 µl of Master Mix to each well.

8. Prepare the Test Inhibitor (10 µl/well): for a titration prepare serial dilutions at concentrations 5-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 5-fold higher than the final desired concentration in TBST.

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

   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 20-fold with TBST Buffer (at this step the compound concentration is 5-fold higher than the desired final concentration). The concentration of DMSO in the dilution is now 5%.

Prepare serial dilutions of the Test Inhibitor at concentrations 5-fold higher than the desired final concentrations using 5% DMSO in TBST Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 5% 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 10 µl of inhibitor solution to the "Test Inhibitor" wells.

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

11. Dilute 4-fold the 4x JMJD2C Direct Assay Buffer with distilled water. This makes 1x JMJD2C Direct Assay Buffer.

12. Thaw **JMJD2C** on ice. Briefly spin the tube containing the enzyme to recover its full content.

13. Dilute JMJD2C to 10 ng/µl in 1x JMJD2C Direct Assay Buffer (20 µl/well).

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

15. Add 20 µl of 1x JMJD2C Direct Assay Buffer to the "Blank" wells.

16. Add 20 µl of diluted JMJD2C solution to the "Positive Control" and "Test Inhibitor" wells.

17. Incubate at RT for 1 hour.

	Blank	**Positive Control**	**Test Inhibitor**
Master Mix	20 µl	20 µl	20 µl
Test Inhibitor	-	-	10 µl
Diluent Solution	10 µl	10 µl	-
Diluted JMJD2C (10 ng/µl)	-	20 µl	20 µl
1x JMJD2C 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.

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.
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

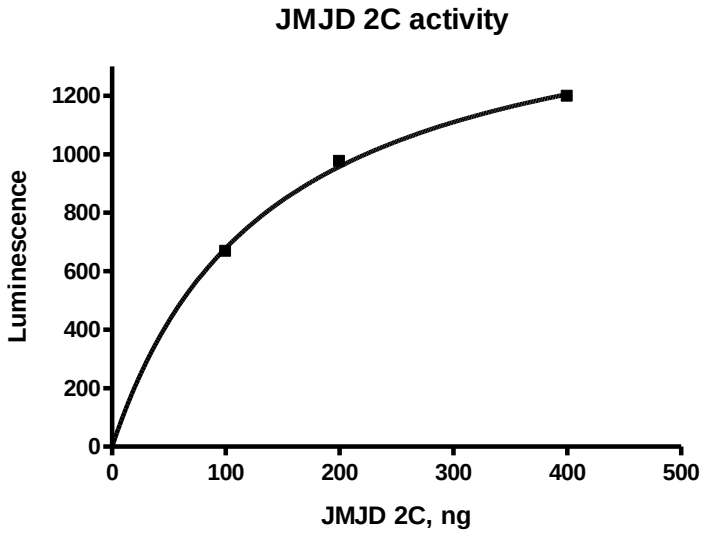

Figure 2: JMJD2C enzyme activity.
JMJD2C enzyme activity was measured using increasing amounts of enzyme. Luminescent intensity was measured using a Bio-Tek fluorescent microplate reader.

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.
Zhu Q., *et al.*, 2022 *Cell Death Discovery* 8:227.

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 (KDM4B) Homogeneous Assay Kit	50414	384 reactions
JMJD2C (KDM4C) Homogeneous Assay Kit	50415	384 reactions
JMJD2D (KDM4D) Homogeneous Assay Kit	79838	384 reactions
JMJD2E (KDM4DL) Homogeneous Assay Kit	50417	384 reactions
JMJD2B (KDM4B), FLAG-tag Recombinant	50104	100 µg

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