Description

The JMJD2B (KDM4B) Homogeneous Assay Kit is designed to measure JMJD2B (Jumonji domain-containing protein 2B) activity for screening and profiling applications. The JMJD2B (KDM4B) Homogeneous Assay Kit comes in a convenient 96-well AlphaLISA® format, with enough purified recombinant JMJD2B (amino acids 2-500), substrate, primary antibody, assay and detection buffer for 100 enzyme reactions.

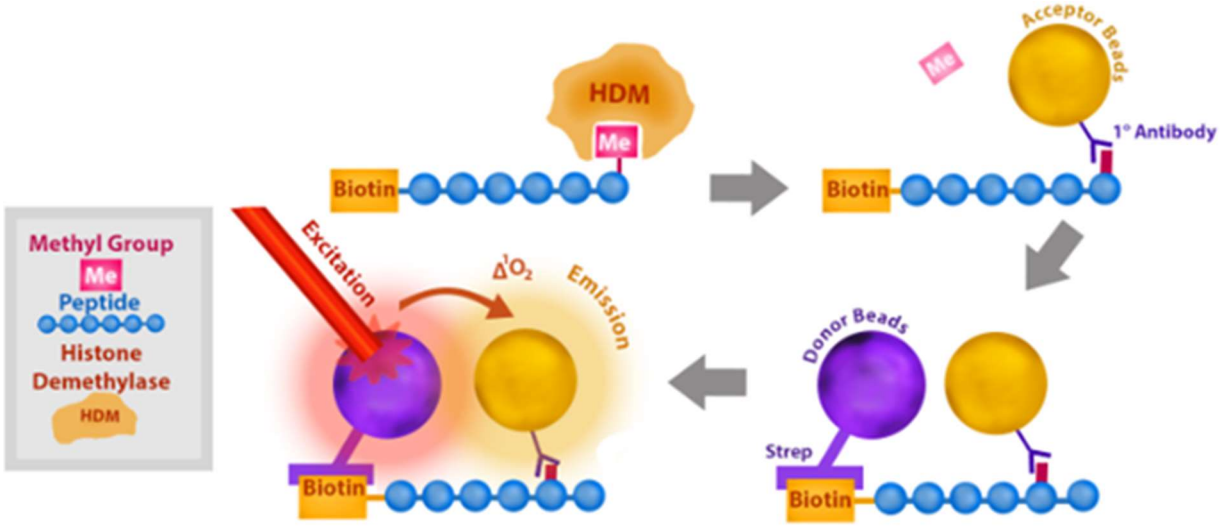

Figure 1: JMJD2B (KDM4B) Homogenous Assay Kit schematic.

A sample containing JMJD2B is incubated with a substrate. This is followed by the addition of acceptor beads and primary antibody, and finally donor beads. Alpha-counts are then counted. Alpha-counts are proportional to JMJD2B demethylase activity.

Background

JMJD2B (Jumonji domain -containing protein 2B), also known as KDM4B, belongs to the JMJD2 subfamily of JMJD histone lysine demethylase proteins. It is involved in demethylating H3K9me2/3, thus participating in osteogenic differentiation of mesenchymal stem cells, regulation of transcription for maintenance of trophoblast stem cells, DNA damage response (DDR), cell cycle progression and apoptosis. JMJD2B is found at high levels in different cancer types, such as breast, gastric and colorectal cancer and Hodgin's lymphoma, and also during inflammation. JMJD2B is regulated by HIF1α (hypoxia-inducible factor 1 alpha), and silencing JMJD2B in colorectal cancer cells can stimulate DDR responses and inhibit cancer cell proliferation. The use of JMJD2B inhibitors is an area of interest that requires further development, and the development of active inhibitors will be beneficial in the cancer therapy field.

Application(s)

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

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Supplied Materials

Catalog #	Name	Amount	Storage
50111	JMJD2B (KDM4B), GST-Tag*	10 µg	-80°C
52140E	Primary Antibody 5	5 µl	-80°C
79841	Biotinylated Histone H3 Peptide Substrate	300 reactions	-80°C
52407	4x HDM Assay Buffer 2	3 x 1 ml	-80°C
52301	4x Detection Buffer	2 ml	-20°C

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

Materials Required but Not Supplied

Name	Ordering Information
AlphaLISA® anti-mouse IgG acceptor beads, 5 mg/ml	Perkin Elmer #AL105C
AlphaScreen® Streptavidin-conjugated donor beads, 5 mg/ml	Perkin Elmer #6760002S
Optiplate - 384	Perkin Elmer #6007290
AlphaScreen® microplate reader	

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 JMJD2B Homogenous Assay Kit is compatible with up to 1% final DMSO concentration.
- Avoid green and blue dyes that absorb light in the AlphaScreen signal emission range (λ=520-620 nm), such as Trypan Blue.
- Avoid the use of potent singlet oxygen quenchers such as sodium azide (NaN_3) or metal ions (Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+} and Ni^{2+}).
- 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. Media like MEM, which lacks these components, does not affect AlphaScreen assays.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include “Blank”, “Positive Control” and “Test inhibitor”.
- We recommend maintaining the diluted protein on ice during use.

- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- Depending on the nature of the inhibitor, pre-incubation of the enzyme with the inhibitor may be necessary.
- 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. Add 750 µl of distilled water to the tube containing the lyophilized Biotinylated Histone H3 Peptide Substrate.

2. Diluted 4-fold the 4x HDM Assay Buffer 2 with distilled water.

3. Prepare the Test Inhibitor (6 µl/well): for a titration prepare serial dilutions at concentrations 3.3-fold higher than the desired final concentrations. The final volume of the reaction is 20 µl.

   2.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in 1x HDM Assay Buffer 2, 3.3-fold more concentrated than the desired final concentrations.

   For the positive and negative controls, use 1x HDM Assay Buffer 2 (Diluent Solution).

   OR

   2.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 30-fold in 1x HDM Assay Buffer 2 to prepare the highest concentration of the 3.3-fold intermediate dilutions. The concentration of DMSO is now 3.3%.

   Prepare serial dilutions of the Test Inhibitor at 3.3-fold the desired final concentrations using 3.3% DMSO in 1x HDM Assay Buffer 2 to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 3.3% DMSO in 1x HDM Assay Buffer 2 (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

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

4. Add 6 µl of inhibitor solution to each well designated “Test Inhibitor”.

5. Add 6 µl of Diluent Solution to the “Blank” and “Positive Control” wells.

6. Thaw JMJD2B on ice. Briefly spin the tube containing enzyme to recover the full content of the tube.

7. Dilute JMJD2B to 5 ng/µl with 1x HDM Assay Buffer 2 (6 µl/well).

8. Add 6 µl of diluted JMJD2B to the “Test Inhibitor” and “Positive Control” wells.

9. Add 6 µl of 1x HDM Assay Buffer 2 to the "Blank" wells.

10. Pre-incubate for 30 minutes at Room Temperature (RT).

11. Prepare a Master Mix (8 µl/well): N wells x (5 µl of 4x HDM Assay Buffer 2 + 3 µl of resuspended Biotinylated Histone H3 Peptide Substrate).

12. Initiate the reaction by adding 8 µl of Master Mix to all wells.

13. Incubate at RT for 1 hour with slow agitation.

Protect your samples from direct exposure to light for steps 2 and 3. Photobleaching will occur!

Component	Test Inhibitor	Blank	Positive Control
Test Inhibitor	6 µl	-	-
Diluent Solution	–	6 µl	6 µl
Diluted JMJD2B (25 ng/µl)	6 µl	-	6 µl
1x HDM Assay Buffer 2	-	6 µl	-
Master Mix	8 µl	8 µl	8 µl
Total	**20 µl**	**20 µl**	**20 µl**

Step 2:

1. Dilute 4-fold the 4X Detection Buffer with distilled water. This makes 1X Detection Buffer.

   Note: Prepare only enough to perform the assay. Store remaining 4x Detection Buffer at -20°C.

2. Dilute anti-mouse acceptor beads diluted 500-fold and Primary Antibody 5 200-fold, together, in 1X Detection Buffer (10 µl of mix/well). Mix well.

3. Add 10 µl of mix to each well.

4. Incubate 30 minutes at RT.

Step 3:

1. Dilute streptavidin-conjugated donor beads 125-fold with 1x Detection Buffer (10 µl/well).

2. Add 10 µl of diluted donor beads to each well.

3. Incubate for 30 minutes at RT in a rotating platform.

4. Read Alpha-Counts.

5. The "Blank" control might be important to determine the background A-screen counts in the assay. The blank value should be subtracted from all other values.

Example Results

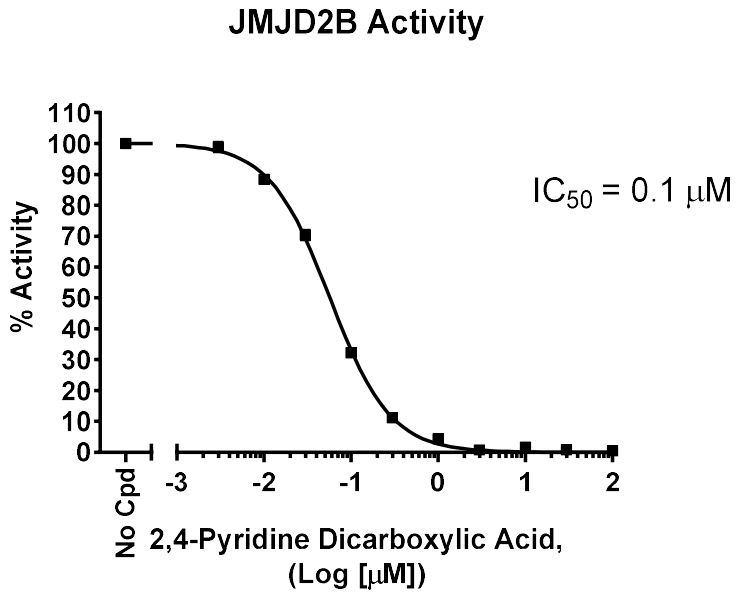

Figure 2: Inhibition of JMJD2B activity by 2, 4-Pyridine Dicarboxylic Acid.
JMJD2B activity was measured in the presence of increasing concentrations of 2, 4-Pyridine Dicarboxylic Acid.

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

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

References
Manni W., *et al.*, 2022 *Signal Transduct Target Ther* 7:304.
Mak K., *et al.*, 2021 *Scientific Reports* 11:884.
Chen L., *et al.*, 2014 *British Journal of Cancer* 110:1014-1026.

Related Products

Products	*Catalog #*	*Size*
JMJD2A (KDM4A) Homogeneous Assay Kit	50413	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
Anti-JMJD2B Polyclonal Antibody	25297	100 µl

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