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Data Sheet ***JMJD1A Homogeneous Assay Kit*** Catalog #50412-2

DESCRIPTION: The *JMJD1A Homogeneous Assay Kit* is designed to measure JMJD1A activity for screening and profiling applications. JMJD1A, also known as JHDM2A and KDM3A, is a JumonjiC (JmjC) domain containing histone lysine demethylase that exhibits demethylation activity toward H3-K₉Me² and H3-K₉Me¹. The *JMJD1A Homogeneous Assay Kit* comes in a convenient AlphaLISA[®] format (Scheme 1), with biotinylated histone H3 peptide substrate, primary antibody, demethylase assay buffer, and purified JMJD1A for 384 enzyme reactions. The key to the *JMJD1A Homogeneous 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 JMJD1A enzyme is incubated with the biotinylated substrate. Next, acceptor beads and primary antibody are added, then donor beads, followed by reading the Alpha-counts.

COMPONENTS:

| Catalog # | Component | Amount | Storage | |
|-----------|---|----------|---------|--|
| 50130 | JMJD1A (KDM3A) | 20 µg | -80°C | (Avoid freeze/thaw cycles!) |
| 52140Q4 | Primary antibody 17-4 | 20 µl | -80°C | |
| 79845 | Biotinylated histone H3 peptide substrate | 500 rxns | -80°C | |
| 52407 | 4x HDM Assay Buffer 2 | 3 ml | -80°C | |
| 52031 | 4x Detection buffer | 2 ml | -20°C | |

MATERIALS REQUIRED BUT NOT SUPPLIED:

AlphaLISA[®] anti-RlgG acceptor beads, 5 mg/ml (PerkinElmer #AL104C)
AlphaScreen[®] Streptavidin-conjugated donor beads, 5 mg/ml (PerkinElmer #6760002S)
Optiplate -384 (PerkinElmer #6007290)
AlphaScreen[®] microplate reader
Adjustable micropipettor and sterile tips

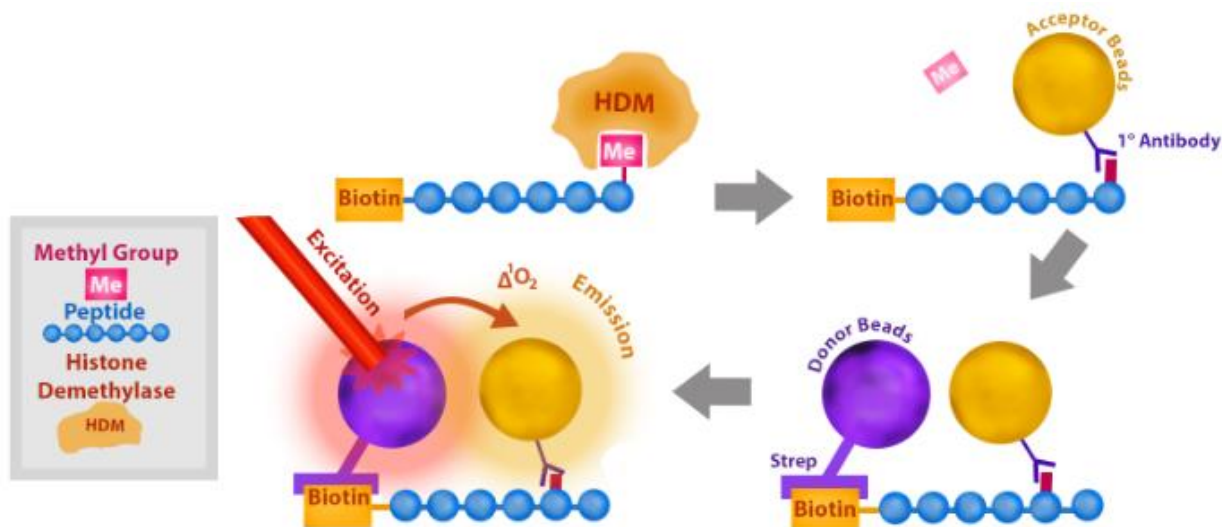
APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

SAFETY: This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous. Do not ingest, inhale, get in eyes, on skin, or on clothing. If so, wash thoroughly.

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Scheme 1: Our histone demethylase assays utilize highly specific antibodies that recognize demethylated products. First, a sample containing the enzyme is incubated with a biotinylated substrate. Next, acceptor beads and primary antibody are added, then donor beads, followed by reading the Alpha-counts, as shown below.



CONTRAINDICATIONS: Green and blue dyes that absorb light in the AlphaScreen signal emission range (520-620 nm), such as Trypan Blue. Avoid the use of the 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. MEM, which lacks these components, does not affect AlphaScreen assays.

STABILITY: At least one year from date of receipt when stored as directed.

REFERENCE(S):

1. Horton, J.R., *et al. Nature Structural & Molecular Biology* 2010; **17(1)**: 38-43.

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate. We recommend preincubating the enzyme with inhibitor, however, it is acceptable to add the substrate mixture and inhibitor followed by diluted JMJD1A without the preincubation step.

Step 1:

- 1) Re-suspend lyophilized **Biotinylated histone H3 peptide substrate** in 500 μ l of distilled water.
- 2) Prepare serial dilutions of the test inhibitors in **1x HDM Assay Buffer 2** (Scheme 2). Add 3 μ l of inhibitor solution to each well designated "Test Sample". For the wells designated "Blank" and "Positive Control" add 3 μ l of the same solution without inhibitor (typically **1x HDM Assay Buffer 2** with respective concentration of DMSO).
- 3) Thaw **JMJD1A** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **JMJD1A** enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: JMJD1A is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*
- 4) Dilute **JMJD1A** in **1x HDM Assay Buffer 2** at 10 ng/ μ l (40 ng/4 μ l). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.
- 5) Preincubate 4 μ l of diluted **JMJD1A** with 3 μ l of diluted inhibitor(s) for up to 30 minutes at room temperature, with slow shaking. For the wells designated as "Blank", add 4 μ l **1x HDM Assay Buffer 2**.
- 6) Prepare master mix: N wells \times (1.5 μ l **4x HDM Assay Buffer 2** + 1 μ l **Biotinylated substrate** + 0.5 μ l **distilled water**).
- 7) Initiate reaction by adding 3 μ l of master mix prepared as described above. Incubate at room temperature for one hour. *Note: All incubations are done with slow shaking on a rotator platform.*

Scheme 2: The serial dilution of the compounds was first performed in 100% DMSO with the highest concentration at (X) mM. Each intermediate compound dilution (in 100% DMSO) will then get directly diluted 30x fold into **1x HDM Assay Buffer 2** for 3.3x concentration (DMSO). From this intermediate step, 3 μ l of compound is added to 4 μ l of demethylase enzyme dilution is incubated for 30 minutes at room temperature. After this incubation, 3 μ l of peptide substrate is added. The final DMSO concentration is 1% for all wells.

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| Reagent | Blank | Positive Control | Test Inhibitor |
|-----------------------------------|-----------------------------|-----------------------------|-----------------------------|
| 1x HDM Assay Buffer 2 | 4 μ l | – | – |
| 4x HDM Assay Buffer 2 | 1.5 μ l | 1.5 μ l | 1.5 μ l |
| Biotinylated Substrate | 1 μ l | 1 μ l | 1 μ l |
| Distilled water | 0.5 μ l | 0.5 μ l | 0.5 μ l |
| Test Inhibitor/Activator | – | – | 3 μ l |
| 1x HDM Assay Buffer 2 (3.3% DMSO) | 3 μ l | 3 μ l | – |
| JMJD1A (10 ng/ μ l) | – | 4 μ l | 4 μ l |
| Total | 10 μl | 10 μl | 10 μl |

Step 2:

Note: Protect your samples from direct exposure to light!

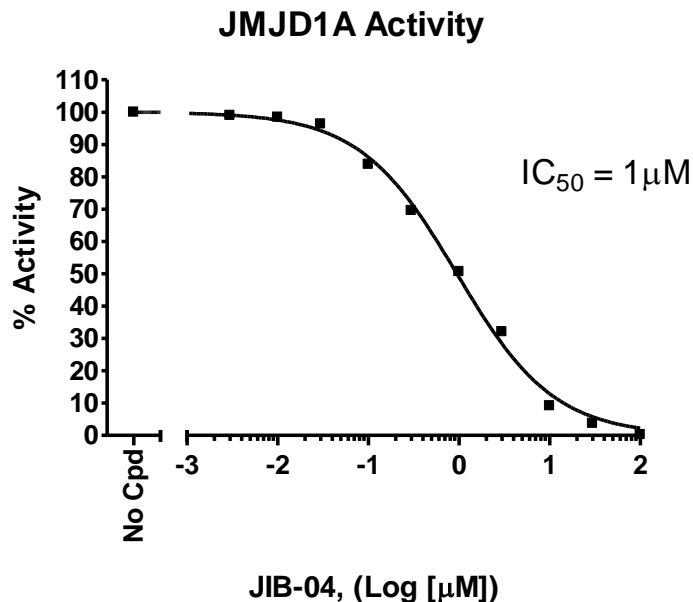
- 1) Dilute anti-Rabbit Acceptor beads (PerkinElmer #AL104C) (1:500) and Primary antibody 17-4 (1:200) with 1x Detection buffer in one step. Add 10 μ l of acceptor beads/antibody mixture per well. Incubate 30 min at room temperature.

Step 3:

- 1) Dilute **Streptavidin-conjugated donor beads** (PE #6760002S) 125-fold with **1x Detection buffer**. Shake on a rotator platform for 30 minutes at room temperature.
- 2) Read Alpha-counts.

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Example of Assay Results:


JMJD1A enzyme activity, measured using the *JMJD1A Homogeneous Assay Kit*, BPS Bioscience Cat. #50412-2. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com

RELATED PRODUCTS:

| <u>Product Name</u> | <u>Catalog #</u> | <u>Size</u> |
|-------------------------------------|------------------|---------------|
| JMJD1B Assay Kit, Homogeneous | 79839 | 384 reactions |
| JHDM1D Assay Kit, Homogeneous | 50420-2 | 384 reactions |
| PHF8 Assay Kit, Homogeneous | 50515-2 | 384 reactions |
| JMJD1A Assay Kit, Homogeneous | 50412-1 | 96 reactions |
| JHDM1D Assay Kit, Homogeneous | 50420-1 | 96 reactions |
| PHF8 Assay Kit, Homogeneous | 50515-1 | 96 reactions |
| JHDM1D Assay Kit, Chemiluminescence | 50612 | 96 reactions |
| JMJD1A recombinant protein | 50130 | 20 μ g |
| JMJD1B recombinant protein | 50421 | 20 μ g |
| JHDM1D recombinant protein | 50419 | 20 μ g |
| PHF8 recombinant protein | 50131 | 20 μ g |

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TROUBLESHOOTING GUIDE

| Problem | Possible Cause | Solution |
|--|---|--|
| Alpha-counts signal of positive control reaction is same as "blank" value. | JMJD1A has lost activity | Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh JMJD1A, BPS Bioscience #50130. Store enzyme in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration. |
| | Streptavidin Donor beads or anti-rIgG acceptor beads fail to show significant signal. | Reorder Streptavidin Donor beads or anti-rIgG acceptor beads from Perkin Elmer. |
| | Incorrect settings on instruments | Refer to instrument instructions for correct settings to increase sensitivity of light detection. |
| Alpha-counts 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. |

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