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# Data Sheet
# *JHDM1D (KDM7A) Homogeneous Assay Kit*
**Catalog # 50420**

**DESCRIPTION:**
The *JHDM1D Homogeneous Assay Kit* is designed to measure activity of JHDM1D for screening and profiling applications. JHDM1D, also known as KDM7A and KIAA1718, is a JumonjiC (JmjC) domain-containing histone lysine demethylase that exhibits demethylation activity toward dimethyl-lysine 9 and dimethyl-lysine 27 on histone H3 (H3K9me2 and H3K27me2 respectively). The *JHDM1D Homogeneous Assay Kit* comes in a convenient AlphaLISA® format, with biotinylated histone H3 peptide substrate, primary antibody, demethylase assay buffer, and purified JHDM1D for 384 enzyme reactions. The key to the *JHDM1D 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 demethylase activity detection. First, a sample containing JHDM1D 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 | |
|---|---|---|---|---|
| 50419 | JHDM1D (KDM7A) | 40 µg | -80°C | ***Avoid Freeze/ Thaw Cycles*** |
| 52140Q3 | Primary antibody 17-4 | 20 µl | -80°C | |
| | Biotinylated histone H3 peptide substrate | 500 rxns | -80°C | |
| 52409 | 4x HDM Assay Buffer 4* | 2 x 1 ml | -80°C | |
| 52410 | 4x HDM Incomplete Assay Buffer | 1 ml | -80°C | |
| | 4x Detection Buffer | 2 ml | -20°C | |

**MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:**
AlphaLISA anti-rIgG 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.

"*Note: *The buffer in this kit was reformulated in May of 2015 with a different reducing agent to improve assay performance. The old formulation can still be purchased upon special request."*

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**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 culture medium RPMI 1640 at >1% leads to 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 6 months from date of receipt when stored as directed.

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

**ASSAY PROTOCOL:**
***All samples and controls should be tested in duplicate.***

**Step 1:**

1) Re-suspend **Biotinylated substrate** in 500 µl of distilled water.

2) Prepare master mix: N wells × (2.5 µl **4x HDM Assay Buffer 4** + **1 µl Biotinylated substrate** + 0.5 µl distilled water).

3) Add 4 µl of master mixture to each well designated for the “Positive Control” and “Test Inhibitor”. For the "Blank", add 2.5 µl **4x HDM Incomplete Assay Buffer** + 1 µl **Biotinylated substrate** + 0.5 µl water.

4) Add 3 µl of inhibitor solution to each well designated “Test Inhibitor”. For the wells designated “Positive Control" and "Blank," add 3 µl of the same solution without inhibitor (Inhibitor buffer).

| Reagent | Blank | Positive Control | Test Inhibitor |
|---|---|---|---|
| 4x HDM Assay Buffer 4 | – | 2.5 µl | 2.5 µl |
| 4x HDM Incomplete Assay Buffer | 2.5 µl | – | – |
| Biotinylated Substrate | 1 µl | 1 µl | 1 µl |
| Distilled water | 0.5 µl | 0.5 µl | 0.5 µl |
| Test Inhibitor/Activator | – | – | 3 µl |
| Inhibitor buffer (no inhibitor) | 3 µl | 3 µl | – |
| JHDM1D (30 ng/µl) | 3 µl | 3 µl | 3 µl |
| **Total** | **10 µl** | **10 µl** | **10 µl** |

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5) Thaw **JHDM1D** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **JHDM1D** enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: **JHDM1D** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

6) Prepare **1x HDM Incomplete Assay Buffer** by diluting **4x HDM Incomplete Assay Buffer** with distilled water. Dilute only the amount required for the assay.

7) Dilute **JHDM1D** in **1x HDM Incomplete Assay Buffer** at 30 ng/µl (90 ng/3 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use. *Note: The incomplete buffer, which does not contain α-ketoglutarate, provides a more accurate background value than a no-enzyme control.*

8) Initiate reaction by adding 3 µl of diluted **JHDM1D** prepared as described above. Incubate at room temperature for one hour. *Note: All incubations are done with slow shaking on a rotator platform.*

***Note: Protect your samples from direct exposure to light for steps 2 and 3!***

**Step 2:**

1) Prepare **1x Detection Buffer** by diluting **4x Detection Buffer** with distilled water. Dilute only the amount required for the assay.

2) Dilute anti-Rabbit Acceptor beads (PerkinElmer #AL104C) 250-fold with **1x Detection buffer**. Add 5 µl per well. Shake plate briefly.

3) Dilute "**Primary antibody 17-4**" 100-fold with **1x Detection buffer**. Add 5 µl per well. Shake plate. Incubate 30 min at room temperature.

*(Alternatively, dilute anti-Rabbit Acceptor beads (1:500) and Primary antibody 17-4 (1:200) with 1x Detection buffer in one step. Add 10 µL of acceptor beads/antibody mixture per well.)*

**Step 3:**

1) Dilute Streptavidin-conjugated donor beads (PE #6760002S) 125-fold with **1x Detection Buffer**. Add 10 µl per well. Shake on a rotator platform for 10-15 minutes at room temperature.

2) Read Alpha-counts.

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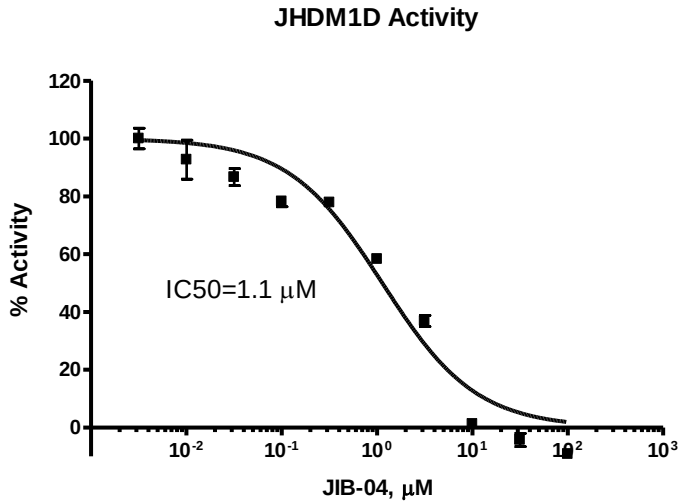
## Example of Assay Results:




Inhibition of JHDM1D enzyme activity by JIB-04, measured using the JHDM1D Homogeneous Assay Kit, BPS Bioscience #50420. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

## RELATED PRODUCTS:

| Product | Cat. # | Size |
|---|---|---|
| JHDM1D recombinant protein | #50419 | 20 µg |
| JMJD1A recombinant protein | #50130 | 20 µg |
| JMJD2A recombinant protein | #50123 | 20 µg |
| JMJD2B recombinant protein | #50104 | 20 µg |
| JMJD2C recombinant protein | #50105 | 20 µg |
| JMJD2D recombinant protein | #50117 | 20 µg |
| JMJD2E recombinant protein | #50118 | 20 µg |
| JMJD3 recombinant protein | #50115 | 20 µg |
| JMJD2A Homogeneous Assay Kit | #50413 | 384 reactions |
| JMJD2B Homogeneous Assay Kit | #50414 | 384 reactions |
| JMJD2C Homogeneous Assay Kit | #50415 | 384 reactions |
| JMJD2E Homogeneous Assay Kit | #50417 | 384 reactions |
| JMJD3 Homogeneous Assay Kit | #50416 | 384 reactions |

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