**Description**

The JHDM1D Homogeneous Assay Kit is designed to measure JHDM1D (Jumonji C domain-containing histone demethylation protein 1D) activity for screening and profiling applications. The JHDM1D Homogeneous Assay Kit comes in a convenient 96-well AlphaLISA® format, with enough purified recombinant JHDM1D (amino acids 2-488), substrate, primary antibody, assay and detection buffer for 100 enzyme reactions.

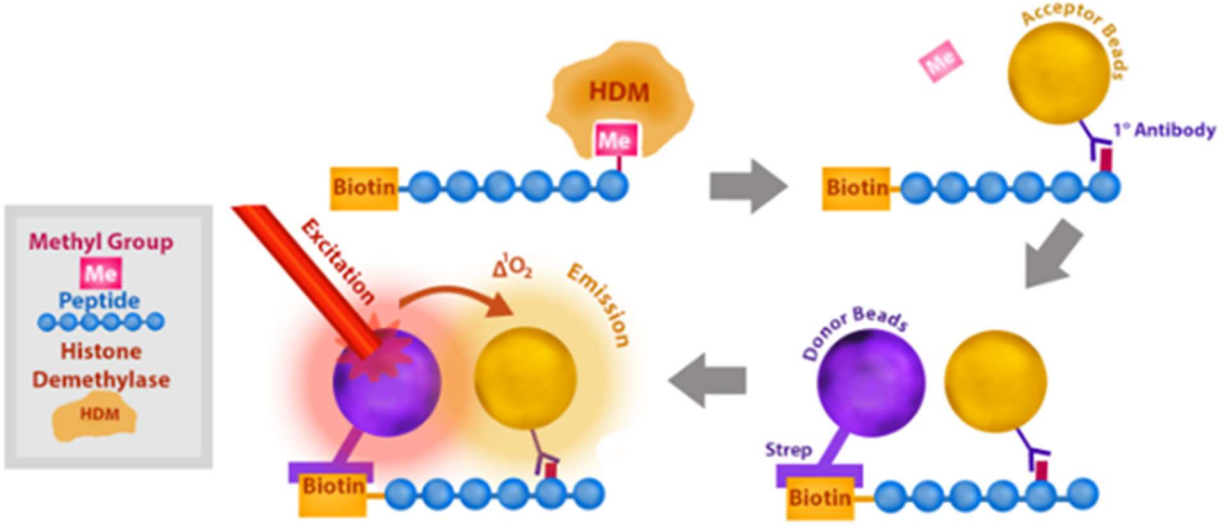


*Figure 1: JHDM1D Homogenous Assay Kit schematic.*

A sample containing JHDM1D is incubated with a biotinylated 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 JHDM1D demethylase activity.

**Background**

JHDM1D (Jumonji C domain-containing histone demethylation protein 1D), also known as KIAA1718 and KDM7A (lysine demethylase 7A), is a JumonjiC (JmjC) domain containing histone lysine demethylase with demethylation activity toward H3-K9Me2 and H3-K27Me2. They are Fe(II) and α-ketoglutarate (2OG) dependent, producing carbon dioxide, succinate and unmethylated protein. By demethylating H3-K9Me2 and H3-K27Me2, which are inhibitory when in their methylated form, JHDM1D releases the brake on transcription and allows for expression of genes involved in multiple biological processes, namely embryonic development, neurogenesis and lipid metabolism. It is linked to tumorigenesis, immune related diseases and neurological and developmental disorders. Multiple inhibitors of JHDM1D inhibitors have been developed, aimed at cancer and osteoarthritis treatment. Drug development targeting JHDM1D is an area of interest, and the development of specific 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.

**Supplied Materials**

| Catalog # | Name | Amount | Storage |
|---|---|---|---|
| 50419 | JHDM1D (KDM7A), FLAG-Tag* | 5 µg | -80°C |
| 52140Q4 | Primary Antibody 17-4 | 5 µl | -80°C |
| 79845 | Biotinylated Histone H3 Peptide Substrate | 300 reactions | -80°C |
| 52409 | 4x HDM Assay Buffer 4 | 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-rabbit IgG acceptor beads, 5 mg/ml | Perkin Elmer #AL104C |
| 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 JHDM1D 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 Daminozide 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 953 µl of distilled water to the tube containing the lyophilized Biotinylated Histone H3 Peptide Substrate.

2. Diluted 4-fold the 4x HDM Assay Buffer 4 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.

   3.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in 1x HDM Assay Buffer 4, 3.3-fold more concentrated than the desired final concentrations.

   For the positive and negative controls, use 1x HDM Assay Buffer 4 (Diluent Solution).

   ***OR***

   3.2 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 4 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 4 to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 3.3% DMSO in 1x HDM Assay Buffer 4 (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 JHDM1D on ice. Briefly spin the tube containing enzyme to recover the full content of the tube.

7. Dilute JHDM1D to 4 ng/µl with 1x HDM Assay Buffer 4 (6 µl/well).

8. Add 6 µl of diluted JHDM1D to the “Test Inhibitor” and “Positive Control” wells.

9. Add 6 µl of 1x HDM Assay Buffer 4 to the “Blank” wells.

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

11. Prepare a Master Mix (8 µl/well): N wells x (5 µl of 4x HDM Assay Buffer 4 + 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 step 2 and 3. Photobleaching will occur!**

| Component | Test Inhibitor | Blank | Positive Control |
|---|---|---|---|
| Test Inhibitor | 6 µl | - | - |
| Diluent Solution | – | 6 µl | 6 µl |
| Diluted JHDM1D (4 ng/µl) | 6 µl | - | 6 µl |
| 1x HDM Assay Buffer 4 | - | 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-rabbit acceptor beads diluted 500-fold and the Primary Antibody 17-4 200-fold, together in one step, 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**

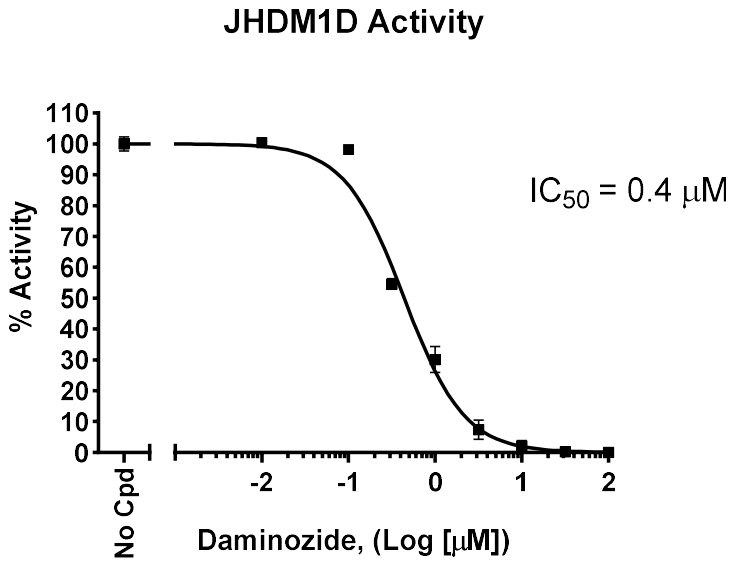


*Figure 2: Inhibition of JHDM1D activity by Daminozide.*
JHDM1D activity was measured in the presence of increasing concentrations of Daminozide.

*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**
Horton, J.R., *et al.*, 2010 *Nature Structural & Molecular Biology*; 17(1): 38-43.
Li C., *et al.*, 2023 *Biochemical Pharmacology* 216: 115799.

**Related Products**

| *Products* | *Catalog #* | *Size* |
|---|---|---|
| JHDM1D (KDM7B) Chemiluminescence Assay Kit | 50612 | 96 reactions |
| PHF8 (KDM7B) Homogeneous Assay Kit | 50515 | 384 reactions |
| PHF8 (KDM7B) (2-525), FLAG-Tag Recombinant | 50131 | 20 µg |
| PHF8 (KDM7B) Full-length, FLAG-Tag Recombinant | 50132 | 20 µg |
| JMJD2B (KDM4B), FLAG-Tag Recombinant | 50104 | 100 µg |
| Anti-PHF8 Polyclonal Antibody | 25308 | 50 µg |



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