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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 (NaN₃) or metal ions (Fe²⁺, Fe³⁺, Cu²⁺, Zn²⁺ and Ni²⁺). 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 $30\text{ ng}/\mu\text{l}$ ($90\text{ ng}/3\ \mu\text{l}$). 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 $3\ \mu\text{l}$ 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 $5\ \mu\text{l}$ per well. Shake plate briefly.
- 3) Dilute "**Primary antibody 17-4**" 100-fold with **1x Detection buffer**. Add $5\ \mu\text{l}$ 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 $10\ \mu\text{L}$ of acceptor beads/antibody mixture per well.)

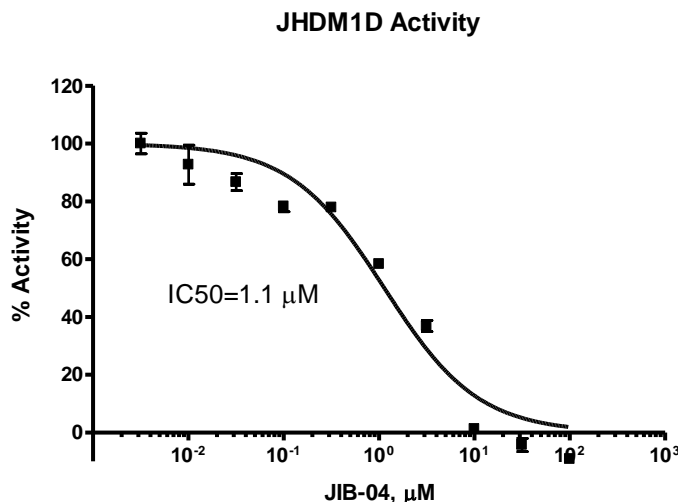
Step 3:

- 1) Dilute Streptavidin-conjugated donor beads (PE #6760002S) 125-fold with **1x Detection Buffer**. Add $10\ \mu\text{l}$ 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:

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
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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