6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

LSD1 Homogeneous Assay Kit

Catalog #50108

DESCRIPTION: The *LSD1 Homogeneous Assay Kit* is designed to measure activity of lysine-specific demethylase (LSD1) for screening and profiling applications. LSD1 is a chromatin-modifying enzyme that specifically removes methyl groups from mono- and dimethylated Lys of histone H3. LSD1 is a critical component of transcriptional regulation via epigenetic histone modifications and is therefore a potential target for drug development. The *LSD1 Homogeneous Assay Kit* comes in a convenient AlphaLISA® format, with biotinylated histone H3 peptide substrate, primary antibody, LSD1 assay buffer, and purified LSD1 for 384 enzyme reactions. The key to the *LSD1 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 detection. First, a sample containing LSD1 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:

Cat. #		Amount	Storage	
50100	LSD1	10 µg	-80°C	***(Avoid freeze/thaw cycles!)***
52140J	Primary antibody 10	40 µl	-80°C	
	Biotinylated histone H3 peptide substrate	400 µl	-80°C	
	3x LSD1 assay buffer 2 (add DTT before use*)	4 ml	-20°C	
	4x Detection buffer	2 ml	-20°C	

***** Adding DTT can affect potency of some inhibitors

MATERIALS REQUIRED BUT NOT SUPPLIED:
DTT (Dithiothreitol), 0.5M (Sigma, Cat. # D0632)
AlphaLISA anti-mIgG acceptor beads, 5 mg/ml (PerkinElmer #AL105C)
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.

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 ($Fe2^+$, $Fe3^+$, $Cu2^+$,

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

$Zn2^+$ and $Ni2^+$). 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.

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

Step 1:
1) Thaw **LSD1** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **LSD1** enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C.
2) Add 30 µl of 0.5 M DTT (not provided) into 4-ml tube with **3x LSD1 assay buffer 2**. Dilute 1 part **3x LSD1 Assay Buffer 2** with 2 parts distilled water (3-fold dilution) to make **1x LSD1 Assay Buffer 2**. Make only a sufficient quantity needed for the assay; store remaining stock solution in aliquots at -20°C.
3) Dilute **LSD1** in **1x LSD1 assay buffer 2** at 5-10 ng/µl. Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.
4) Using master mixes as much as possible, add the following reagents to the microwells, in duplicate:

	Positive Control	**Test Sample**	**Blank**
LSD1 (5-10 ng/µl)	2.5 µl	2.5 µl	–
3x LSD1 assay buffer 2	2.5 µl	2.5 µl	2.5 µl
Biotinylated substrate	1 µl	1 µl	1 µl
Test Inhibitor/Activator	–	X µl	–
H_2O	4 µl	4 – X µl	6.5 µl
Total	**10 µl**	**10 µl**	**10 µl**

4) Add the entire reaction mixture (10 µl) to the well of a 384-well white plate. Incubate at room temperature for 30-45 minutes.

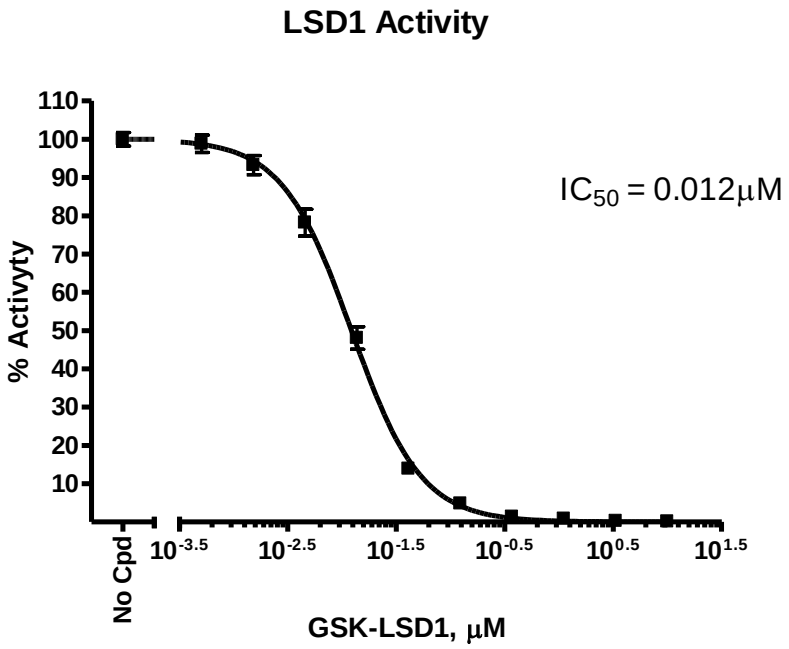
Step 2:
Note: Protect your samples from direct exposure to light!
1) Dilute anti-Mouse Acceptor beads (PerkinElmer #AL105C) 1:250-fold with 1x Detection buffer. Add 5 µl per well. Shake plate briefly.
2) Dilute “Primary antibody 10” 50-fold with 1x Detection buffer. Add 5 µl per well. Shake on a rotator platform for 30 minutes at room temperature.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

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 15 minutes at room temperature.
2) Read Alpha-counts.

Example of Assay Results:



LSD1 enzyme activity, measured using the LSD1 Homogeneous Assay Kit, BPS Bioscience Cat. #50108. *Note: Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

REFERENCES:

1. Forneris, F., Binda, C., Dall'Aglio, A., Fraaije, M.W., Battaglioli, E., and Mattevi, A. *J. Biol. Chem.* 2006; **281(46):**35289-95.
2. Zhou, M., Diwu, Z., Panchuk-Voloshina, N., and Haugland, R.P. *Anal. Biochem.* 1997; **253(2):**162-8.

RELATED PRODUCTS:

LSD1 recombinant protein	#50100	50 µg
LSD1 Fluorescent Assay Kit	#50106	96 reactions
LSD1 Fluorescent Assay Kit	#50107	384 reactions
LSD1 substrate	#50101	500 µl
JMJD2A Homogeneous Assay Kit	#50413	384 reactions

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.
To place your order, please contact us by Phone **1.858.829.3082** Fax **1.858.481.8694**
Or you can Email us at: **info@bpsbioscience.com**
Please visit our website at: **www.bpsbioscience.com**

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

JMJD2B Homogeneous Assay Kit	#50414	384 reactions
JMJD2C Homogeneous Assay Kit	#50415	384 reactions
JMJD2E Homogeneous Assay Kit	#50417	384 reactions
JMJD2C Assay Kit, Chemiluminescent	#50405	96 reactions
JMJD2A recombinant protein	#50103	20 µg
JMJD2B recombinant protein	#50104	20 µg
JMJD2C recombinant protein	#50105	20 µg
JMJD2E recombinant protein	#50118	20 µg

AlphaScreen® and AlphaLISA® are registered trademarks of PerkinElmer, Inc.