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Data Sheet

LSD1 Chemiluminescent Assay Kit

Catalog # 50109

DESCRIPTION: The *LSD1 Chemiluminescent Assay Kit* is designed to directly measure activity of human lysine-specific demethylase (LSD1) enzymes containing LSD1 for screening and profiling applications. LSD1 is a chromatin-modifying enzyme that specifically removes methyl groups from mono- and di-methylated 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 Chemiluminescent Assay Kit* comes in a convenient format, with a 96-well plate precoated with the methylated histone H3 peptide substrate, primary antibody, the secondary HRP-labeled antibody, demethylase assay buffer, and purified LSD1 for 100 enzyme reactions. The key to the *LSD1 Chemiluminescent 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 LSD1 enzyme is incubated with a sample containing assay buffer. Next, primary antibody is added. Finally, the plate is treated with an HRP-labeled secondary antibody followed by addition of the HRP substrate to produce chemiluminescence that can then be measured using a chemiluminescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
50100	LSD1	10 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
52140J	Primary antibody 10	25 µl	-80°C	
52130H	Secondary HRP-labeled antibody 1	10 µl	-80°C	
	3x LSD1 assay buffer 2	3 ml	-20°C	
52100	Blocking buffer	50 ml	+4°C	
	HRP chemiluminescent substrate A (transparent bottle)	6 ml	+4°C	
	HRP chemiluminescent substrate B (brown bottle)	6 ml	+4°C	
	Black microplate precoated with histone substrate	1	+4°C	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
TBST buffer (1 x TBS, pH 8.0, containing 0.05% Tween20)
Luminometer or fluorescent microplate reader capable of reading chemiluminescence
Adjustable micropipettor and sterile tips
Rotating or rocker platform

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

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CONTRAINDICATIONS: DMSO >1%, strong acids or bases, ionic detergents, high salt

STABILITY: One year from date of receipt when stored as directed.

REFERENCES:

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

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

Step 1:

1) Rehydrate the microwells by adding 200 µl of TBST buffer (1 x TBS, pH 8.0, containing 0.05% Tween-20) to every well. Incubate 15 minutes at room temperature. Tap the plate onto clean paper towels to remove liquid.

2) 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. *Note: LSD1 is sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

3) Add 10 µl **3x LSD1 assay buffer 2** + 15 µl distilled **H_2O** to each well.

4) Dilute LSD1 in 1X LSD1 assay buffer 2 at 5 ng/µl. Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

5) Add 5 µl of **Inhibitor** solution of each well designated “Test Inhibitor”. For the “Positive Control" and “Blank”, add 5 µl of the same solution without inhibitor (**Inhibitor buffer**).

6) Add 20 µl of 1X LSD1 assay buffer 2 to the well designated “Blank".

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	Positive Control	Test Inhibitor	Blank
3x LSD1 assay buffer 2	10 µl	10 µl	10 µl
H_2O	15 µl	15 µl	15 µl
Inhibitor	–	5 µl	–
Inhibitor buffer (no inhibitor)	5 µl	–	5 µl
1x LSD1 assay buffer 2	–	–	20 µl
LSD1 (5 ng/µl)	20 µl	20 µl	–
Total	**50 µl**	**50 µl**	**50 µl**

7) Initiate reaction by adding 20 µl of diluted **LSD1** enzyme to the wells designated "Positive Control" and "Test Inhibitor". Incubate at room temperature for 30-45 min.

8) Remove the supernatant from the wells and wash the plate three times with 200 µl TBST buffer. Blot dry onto clean paper towels.

9) Add 100 µl of Blocking buffer to every well. Shake on a rotating platform for 10 min. Remove supernatant as described above.

Step 2:

1) Dilute "**Primary antibody 10**" 400-fold with Blocking buffer.

2) Add 100 µl per well. Incubate 1 hour at room temperature with slow shaking.

3) Remove the supernatant from the wells and wash plate three times with 200 µl TBST buffer and incubate in Blocking buffer as described in steps 1-8 and 1-9.

Step 3:

1) Dilute "**Secondary HRP-labeled antibody 1**" 1,000-fold with Blocking buffer.

2) Add 100 µl per well. Incubate for 30 minutes at room temperature with slow shaking.

3) Remove the supernatant from the wells and wash plate three times with 200 µl TBST buffer and incubate in Blocking buffer as described in steps 1-8 and 1-9.

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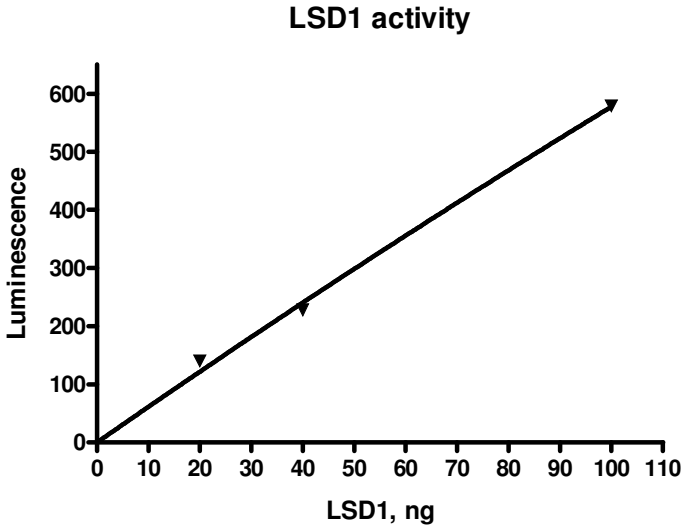
4) Just before use, mix on ice 50 µl HRP chemiluminescent substrate A and 50 µl HRP chemiluminescent substrate B and add 100 µl per well. Discard any unused chemiluminescent reagent after use.
5) Immediately read sample in a luminometer or microtiter-plate reader capable of reading chemiluminescence.

Reading Chemiluminescence:

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavenlength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure you are using your plate reader in a LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Make sure you don't have filter when emit the light (Synergy 2 BioTek: use "hole" position on filter wheel). Optics position – Top. Read type: endpoint. Sensitivity may be adjusted based on luminescence of a control without enzyme (typically we set this value as 100 when using Synergy 2 plate reader).

EXAMPLE OF ASSAY RESULTS:



LSD1 enzyme activity, measured using the LSD1 Chemiluminescent Assay Kit, BPS Bioscience #50109. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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RELATED PRODUCTS:

LSD1 recombinant protein	#50100	50 µg
LSD1 Fluorescent Assay Kit	#50106	96 reactions
LSD1 Fluorescent Assay Kit	#50107	384 reactions
LSD1 Homogeneous Assay Kit	#50108	384 reactions
LSD1 substrate	#50101	500 µl
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
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