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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	12.5 µl	-80°C	
52130H	Secondary HRP-labeled antibody 1	10 µl	-80°C	
	3x LSD1 assay buffer 2 (add DTT before use*)	4 ml	-20°C	
52100	Blocking buffer 4	50 ml	+4°C	
	HRP chemiluminescent substrate A (transparent bottle)	6 ml	+4°C	
	HRP chemiluminescent substrate B (brown bottle)	6 ml	+4°C	
	Microplate precoated with histone substrate	1	+4°C	

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

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
DTT (Dithiothreitol), 0.5M (Sigma, Cat. # D0632)
TBST buffer (1 x TBS, pH 8.0, containing 0.05% Tween20)
Luminometer or microplate reader capable of reading chemiluminescence
Adjustable micropipettor and sterile tips
Rotating or rocker platform

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APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

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 N wells, where N is the total number of wells in the experiment, 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 strip plate onto clean paper towels to remove liquid.

2) Prepare at least (20µL x N) of Assay Buffer + DTT: Dilute 500 mM DTT (not provided) to 3 mM DTT in **3x LSD1 assay buffer 2**. Freeze unused buffer. Prepare master mix: N wells × (10 µl **3x LSD1 Assay Buffer 2** + 15 µl distilled water). Add 25 µl of master mixture to each well.

3) 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). *Note: Keep final DMSO concentration ≤1%.*

	Blank	Positive Control	Test Inhibitor
Master Mixture	25 µl	25 µl	25 µl
Test Inhibitor/Activator	–	–	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**

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4) 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.

5) Add 20 µl **1x LSD1 assay buffer 2** to wells designated as "Blank".

6) 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 very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

7) 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.

8) Initiate reaction by adding 20 µl of **diluted LSD1** prepared as described above to wells designated "Positive Control" and "Test Inhibitor". Incubate at room temperature for one hour.

9) Wash the strip plate three times with TBST buffer. Blot dry onto clean paper towels.

10) Add 100 µl of **Blocking buffer 4** to every well. Shake on a rotating platform for 10 minutes. Remove the supernatant from the wells.

Step 2:

1) Dilute "**Primary antibody 10**" 800-fold with **Blocking buffer 4**.

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

3) Wash strip plate with TBST buffer and incubate in **Blocking buffer 4** as described in step 1-9 and 1-10.

Step 3:

1) Dilute "**Secondary HRP-labeled antibody 1**" 1,000-fold with **Blocking buffer 4**.

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

3) Wash strip plate with TBST buffer and incubate in **Blocking buffer 4** as described in step 1-9 and 1-10.

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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.

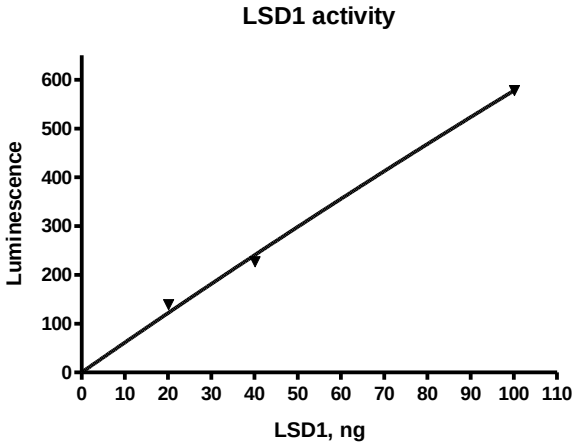
5) Immediately read sample in a luminometer or microtiter-plate reader capable of reading chemiluminescence.

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:

Product Name	Catalog	Size
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

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	LSD1 has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh LSD1, (BPS Bioscience #50100). Store enzyme in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Antibody reaction is insufficient	Increase time for primary antibody incubation. Avoid freeze/thaw cycles of antibodies.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity of light detection.
	Chemiluminescent reagents mixed too soon	Chemiluminescent solution should be used within 15 minutes of mixing. Ensure both reagents are properly mixed.
Luminescent signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
Background (signal to noise ratio) is high	Insufficient washes	Increase number of washes. Increase wash volume. Increase Tween-20 concentration to 0.1% in TBST.
	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of LSD1 (BPS Bioscience #50100) to create a standard curve.