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Data Sheet

LSD1 Homogeneous Assay Kit

Catalog #w60119

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	
w60111	LSD1	10 µg	-80℃	***(Avoid freeze/thaw cycles!)***
w62151J	Primary antibody 10	40 µl	-80℃	
	Biotinylated histone H3 peptide substrate	400 µl	-80℃	
	3x LSD1 assay buffer 2	3 ml	-20℃	
	4x Detection buffer	2 ml	-20℃	

MATERIALS REQUIRED BUT NOT SUPPLIED:
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 ($Fe2^+$, $Fe3^+$, $Cu2^+$, $Zn2^+$ 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.

WEST BIOSCIENCE

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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℃.
2) 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.
3) 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	–
4x 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.

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.

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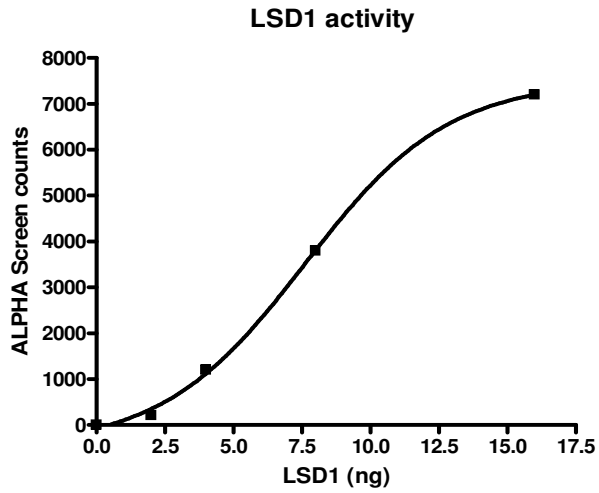
Example of Assay Results:



LSD1 enzyme activity, measured using the LSD1 Homogeneous Assay Kit, West Bioscience Cat. #w60119. *Note: Data shown is lot-specific. For lot-specific information, please contact West Bioscience, Inc. at sale@westbioscience.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.