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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet

LSD2(KDM1B) Homogeneous Assay Kit (Dimethyl)

Catalog #50614

DESCRIPTION: The *LSD2(KDM1B) Homogeneous Assay Kit (Dimethyl)* is designed to measure the activity of lysine-specific demethylase (LSD2) for screening and profiling applications. LSD2, also called KDM1B, is a chromatin-modifying enzyme that specifically removes methyl groups from mono- and dimethylated Lys of histone H3, thereby acting as a corepressor of transcription. LSD2 is a potential target for drug development. The *LSD2 Homogeneous Assay Kit (Dimethyl)* comes in a convenient AlphaLISA® format, with biotinylated, dimethylated histone H3 (2MeK4) peptide substrate, primary antibody to detect mono-methylated product, LSD2 assay buffer, and purified LSD2 for 384 enzyme reactions. The key to the *LSD2 Homogeneous Assay Kit (Dimethyl)* 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 LSD2 enzyme is incubated with the biotinylated substrate. Next, acceptor beads are added, then donor beads, followed by reading the Alpha-counts.

COMPONENTS:

Cat. #		Amount	Storage	
50124	LSD2 (KDM1B)	40 µg	-80°C	***(Avoid freeze/thaw cycles!)***
52140V	Primary antibody 22	25 µl	-80°C	
	Biotinylated histone H3 peptide substrate (2MeK4)	500 rxns	-80°C	
	4x LSD2 assay buffer	3 ml	-20°C	
	4x Detection buffer	2 ml	-20°C	

MATERIALS 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.

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.

STABILITY: At least one year from date of receipt when stored as directed.

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REFERENCES: Binda, C., et al., J. Am. Chem. Soc. 2010; **132(19)**: 6827-6833.
Welte, M., et al., Cell Press. 2005; **15(14)**: 1266-1275.

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

Step 1:

1) Re-suspend lyophilized **Biotinylated histone H3 peptide substrate** in 500 µl of distilled water.

2) Dilute 1 part **4x LSD2 Assay Buffer** with 3 parts distilled water (4-fold dilution) to make **1x LSD2 Assay Buffer**. Dilute **primary antibody 22** 10-fold using **1x LSD2 Assay Buffer**. Make only a sufficient quantity needed for the assay; store remaining undiluted buffer in aliquots at -20°C and undiluted antibody in aliquots at -80°C immediately.

3) Prepare master mix: N wells × (1.5 µl **4x LSD2 assay buffer** + 1 µl **Biotinylated histone H3 peptide substrate** + 0.5 µl **diluted primary antibody 22**). Add 3 µl of master mix to each well.

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).

	Positive Control	Test Sample	Blank
4x LSD2 assay buffer	1.5 µl	1.5 µl	1.5 µl
Biotinylated substrate	1 µl	1 µl	1 µl
Primary antibody 22 (10-fold diluted)	0.5 µl	0.5 µl	0.5 µl
Test inhibitor	–	3	–
Inhibitor buffer (no inhibitor)	3	–	3
1x LSD2 assay buffer	–	–	4 µl
LSD2 (20 ng/µl)	4 µl	4 µl	–
Total	**10 µl**	**10 µl**	**10 µl**

5) Add 4 µl of **1x LSD2 assay buffer** to wells designated as “Blank”.

6) Thaw LSD2 on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot LSD2 enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. Note: LSD2 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.

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7) Dilute **LSD2** in **1x LSD2 assay buffer** at ~20 ng/µl. Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

8) Initiate the reaction by adding 4 µl of **diluted LSD2** prepared as described above to wells designated "Positive Control" and "Test Inhibitor". Incubate at room temperature for one hour. *Note: All incubations are done with slow shaking on a rotator platform.*

Step 2:

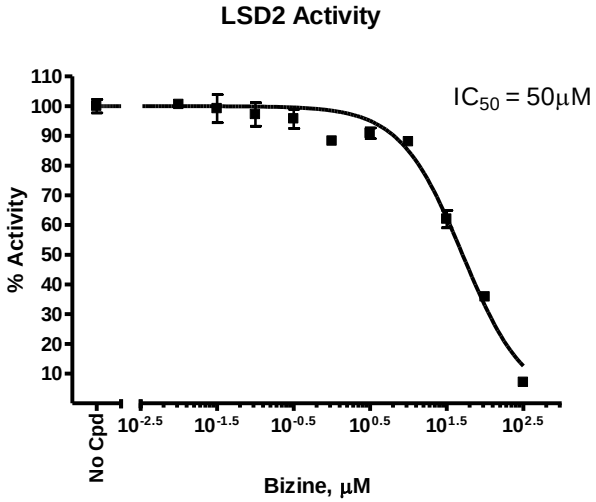
Note: Protect your samples from direct exposure to light!

1) Dilute anti-Rabbit Acceptor beads (PerkinElmer #AL104C) 1:500-fold with 1x Detection buffer. Add 10 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 45 minutes at room temperature.
2) Read Alpha-counts.

Example of Assay Results:



LSD2 enzyme activity, measured using the LSD2 Homogeneous Assay Kit (Dimethyl), BPS Bioscience Cat. #50614. The compound was pre-incubated with the LSD2 enzyme for 30 min before the reaction was initiated with the addition of master mix. *Note: Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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RELATED PRODUCTS:

LSD2 (KDM1B) recombinant protein	#50124	20 µg
LSD1 recombinant protein	#50100	50 µg
LSD2 (KDM1B) Homogeneous Assay Kit (Monomethyl)	#50613	384 reactions
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
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