6044 Cornerstone Court West, Ste. E
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Tel: 1.858.829.3082
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Email: info@bpsbioscience.com

Data Sheet

LSD1 Fluorescent Assay Kit

Catalog # 50106
Size: 96 reactions

DESCRIPTION: Human lysine-specific demethylase (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 Assay Kit* is designed to measure LSD1 activity of purified LSD1 enzymes containing LSD1 using fluorimetry. The assay is based on the analysis of the hydrogen peroxide produced during the demethylation reaction mediated by LSD1.

The *LSD1 Assay Kit* is provided in a convenient 96-well format, with purified LSD1, Amplex Red reagent, peroxidase, and LSD1 assay buffer for 96 enzyme reactions. The key to the *LSD1 Assay Kit* is the methylated peptide substrate. Using this kit, only two simple steps on a microtiter plate are required for LSD1 reactions. First, a sample containing LSD1 is incubated in a reaction mixture with the methylated peptide substrate. Second, peroxidase and Amplex Red reagent are added and fluorescence (λ_{ex}=530±13 nm, λ_{em}=590±18 nm) is measured using a plate reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
50100	LSD1 recombinant enzyme	30 µg	-80°C	***Avoid freeze/ thaw cycles!***
50101	Methylated peptide substrate (200 µM)	500 µl	-80°C	
	Amplex Red reagent (10 mM)	50 µl	-80°C	
	Peroxidase (10 U/ml)	100 µl	-80°C	
	2x LSD1 assay buffer	20 ml	-20°C	
	White 96-well microtiter plate	1	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
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 screening small molecular inhibitors for drug discovery and HTS applications.

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

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2. Zhou, M., et al. *Anal. Biochem.* 1997; **253(2):**162-8.

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

Step 1:

1) Prepare the master mixture: N wells x (15 µl **2x LSD1 assay buffer 1** + 5 µl **Methylated peptide substrate**).

2) Add 20 µl of master mixture to each well designated for the "Positive Control", "Test Inhibitor", and "Blank". For the wells labeled "Substrate Control", add only 15 µl **2x LSD1 assay buffer 1**.

3) Thaw **LSD1 enzyme** 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 enzyme is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

4) Dilute **LSD1 enzyme** in **1X LSD1 assay buffer** at 10–15 ng/µl (100–150 ng/reaction). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

5) Add 10 µl of Inhibitor solution of each well labeled as "Test Inhibitor". For the "Positive Control" , "Substrate Control", and "Blank", add 10 µl of the same solution without inhibitor (Inhibitor buffer).

6) Add 20 µl of **1X LSD1 assay buffer** to the wells designated "Blank". Add 5 µl of **1X LSD1 assay buffer** to the wells designated "Substrate Control".

	Positive Control	Test Inhibitor	Substrate Control	Blank
2x LSD1 assay buffer 1	15 µl	15 µl	15 µl	15 µl
Methylated peptide substrate (200 µM)	5 µl	5 µl	–	5 µl
Test Inhibitor	–	10 µl	–	–
Inhibitor buffer (no inhibitor)	10 µl	–	10 µl	10 µl
1x LSD1 assay buffer 1	–	–	5 µl	20 µl
LSD1 (10–15 ng/µl)	20 µl	20 µl	20 µl	–
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

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7) Initiate reaction by adding 20 µl of diluted **LSD1 enzyme** (10-15 ng/µl) to the wells designated “Positive Control”, "Test Inhibitor", and “Substrate Control”. Incubate for 30 minutes at room temperature.

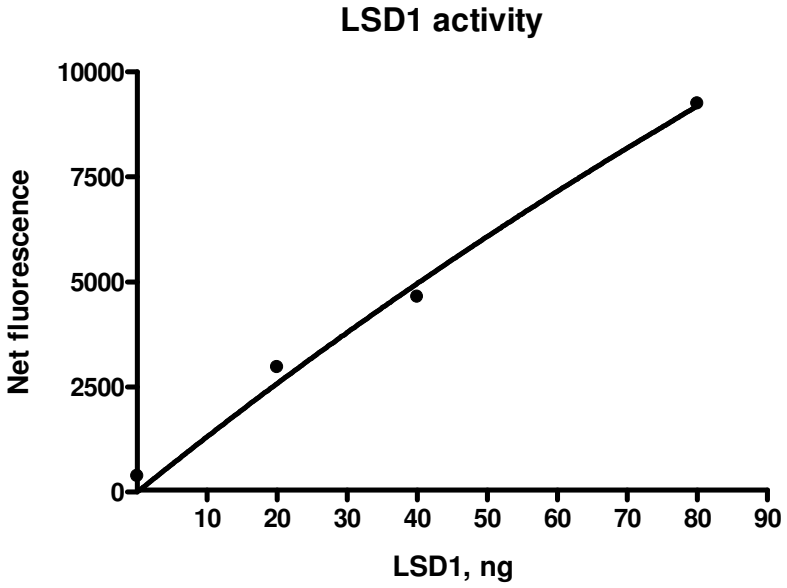
Step 2:

1) Immediately before use: add 50 µl of 10 mM **Amplex Red** and 100 µl of 10 U/ml **Peroxidase** to 4850 µl of **1x LSD1 buffer**. Mix well.

2) Add 50 µl to each microtiter well. Mix well. Incubate at room temperature for 5 minutes.

3) Read fluorescence at λ_{ex}=530 nm and λ_{em}=590 nm. “Blank” value is subtracted from all measurements.

Example of Assay Results:



LSD1 enzyme activity, measured using the *LSD1 Assay Kit*, BPS Bioscience #50106.
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 Assay Kit	#50107	384 reactions
LSD1 recombinant protein	#50100	50 µg
COREST recombinant protein	#50274	100 µg
LSD1 substrate	#50101	500 µl
LSD1 Homogeneous Assay Kit	#50108	384 reactions
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
Fluorescent signal of positive control reaction is weak	LSD1 enzyme has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzyme (LSD1, BPS Bioscience #50100). Store enzyme in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Incorrect wavelength	Use correct filters for fluorescent plate reader. Reading at wavelengths outside of 590 ± 18 nm will give a decreased signal.
	Detection reagents mixed too soon	Amplex Red/peroxidase solution should be used within 15 minutes of mixing. Ensure both reagents are properly mixed.
Fluorescent 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	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 enzyme (LSD1, BPS Bioscience #50100) to create a standard curve.

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LSD1 Fluorescent Assay Kit

Catalog # 50106
Size: 96 reactions

DESCRIPTION: Human lysine-specific demethylase (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 Assay Kit* is designed to measure LSD1 activity of purified LSD1 enzymes containing LSD1 using fluorimetry. The assay is based on the analysis of the hydrogen peroxide produced during the demethylation reaction mediated by LSD1.

The *LSD1 Assay Kit* is provided in a convenient 96-well format, with purified LSD1, Amplex Red reagent, peroxidase, and LSD1 assay buffer for 96 enzyme reactions. The key to the *LSD1 Assay Kit* is the methylated peptide substrate. Using this kit, only two simple steps on a microtiter plate are required for LSD1 reactions. First, a sample containing LSD1 is incubated in a reaction mixture with the methylated peptide substrate. Second, peroxidase and Amplex Red reagent are added and fluorescence (λ_{ex}=530±13 nm, λ_{em}=590±18 nm) is measured using a plate reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
50100	LSD1 recombinant enzyme	30 µg	-80°C	***Avoid freeze/ thaw cycles!***
50101	Methylated peptide substrate (200 µM)	500 µl	-80°C	
	Amplex Red reagent (10 mM)	50 µl	-80°C	
	Peroxidase (10 U/ml)	100 µl	-80°C	
	2x LSD1 assay buffer	20 ml	-20°C	
	White 96-well microtiter plate	1	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
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 screening small molecular inhibitors for drug discovery and HTS applications.

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

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2. Zhou, M., et al. *Anal. Biochem.* 1997; **253(2):**162-8.

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

Step 1:

1) Prepare the master mixture: N wells x (15 µl **2x LSD1 assay buffer 1** + 5 µl **Methylated peptide substrate**).

2) Add 20 µl of master mixture to each well designated for the "Positive Control", "Test Inhibitor", and "Blank". For the wells labeled "Substrate Control", add only 15 µl **2x LSD1 assay buffer 1**.

3) Thaw **LSD1 enzyme** 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 enzyme is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

4) Dilute **LSD1 enzyme** in **1X LSD1 assay buffer** at 10–15 ng/µl (100–150 ng/reaction). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

5) Add 10 µl of Inhibitor solution of each well labeled as "Test Inhibitor". For the "Positive Control" , "Substrate Control", and "Blank", add 10 µl of the same solution without inhibitor (Inhibitor buffer).

6) Add 20 µl of **1X LSD1 assay buffer** to the wells designated "Blank". Add 5 µl of **1X LSD1 assay buffer** to the wells designated "Substrate Control".

	Positive Control	Test Inhibitor	Substrate Control	Blank
2x LSD1 assay buffer 1	15 µl	15 µl	15 µl	15 µl
Methylated peptide substrate (200 µM)	5 µl	5 µl	–	5 µl
Test Inhibitor	–	10 µl	–	–
Inhibitor buffer (no inhibitor)	10 µl	–	10 µl	10 µl
1x LSD1 assay buffer 1	–	–	5 µl	20 µl
LSD1 (10–15 ng/µl)	20 µl	20 µl	20 µl	–
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

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7) Initiate reaction by adding 20 µl of diluted **LSD1 enzyme** (10-15 ng/µl) to the wells designated “Positive Control”, "Test Inhibitor", and “Substrate Control”. Incubate for 30 minutes at room temperature.

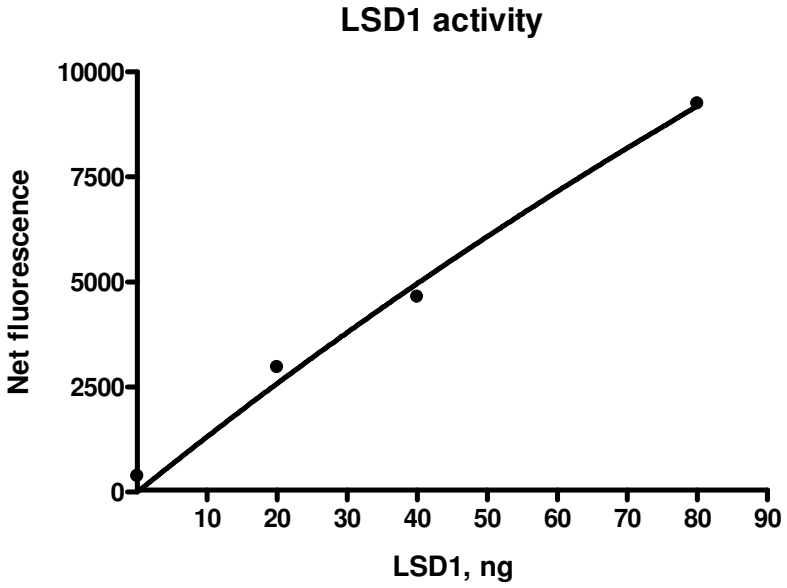
Step 2:

1) Immediately before use: add 50 µl of 10 mM **Amplex Red** and 100 µl of 10 U/ml **Peroxidase** to 4850 µl of **1x LSD1 buffer**. Mix well.

2) Add 50 µl to each microtiter well. Mix well. Incubate at room temperature for 5 minutes.

3) Read fluorescence at λ_{ex}=530 nm and λ_{em}=590 nm. “Blank” value is subtracted from all measurements.

Example of Assay Results:



LSD1 enzyme activity, measured using the *LSD1 Assay Kit*, BPS Bioscience #50106.
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Product Name	Catalog #	Size
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LSD1 recombinant protein	#50100	50 µg
COREST recombinant protein	#50274	100 µg
LSD1 substrate	#50101	500 µl
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JMJD2A Homogeneous Assay Kit	#50413	384 reactions
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	Incorrect wavelength	Use correct filters for fluorescent plate reader. Reading at wavelengths outside of 590 ± 18 nm will give a decreased signal.
	Detection reagents mixed too soon	Amplex Red/peroxidase solution should be used within 15 minutes of mixing. Ensure both reagents are properly mixed.
Fluorescent 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	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 enzyme (LSD1, BPS Bioscience #50100) to create a standard curve.

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COMPONENTS:

Catalog #	Component	Amount	Storage	
50100	LSD1 recombinant enzyme	30 µg	-80°C	***Avoid freeze/ thaw cycles!***
50101	Methylated peptide substrate (200 µM)	500 µl	-80°C	
	Amplex Red reagent (10 mM)	50 µl	-80°C	
	Peroxidase (10 U/ml)	100 µl	-80°C	
	2x LSD1 assay buffer	20 ml	-20°C	
	White 96-well microtiter plate	1	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
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 screening small molecular inhibitors for drug discovery and HTS applications.

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ASSAY PROTOCOL:

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Step 1:

1) Prepare the master mixture: N wells x (15 µl **2x LSD1 assay buffer 1** + 5 µl **Methylated peptide substrate**).

2) Add 20 µl of master mixture to each well designated for the "Positive Control", "Test Inhibitor", and "Blank". For the wells labeled "Substrate Control", add only 15 µl **2x LSD1 assay buffer 1**.

3) Thaw **LSD1 enzyme** 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 enzyme is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

4) Dilute **LSD1 enzyme** in **1X LSD1 assay buffer** at 10–15 ng/µl (100–150 ng/reaction). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

5) Add 10 µl of Inhibitor solution of each well labeled as "Test Inhibitor". For the "Positive Control" , "Substrate Control", and "Blank", add 10 µl of the same solution without inhibitor (Inhibitor buffer).

6) Add 20 µl of **1X LSD1 assay buffer** to the wells designated "Blank". Add 5 µl of **1X LSD1 assay buffer** to the wells designated "Substrate Control".

	Positive Control	Test Inhibitor	Substrate Control	Blank
2x LSD1 assay buffer 1	15 µl	15 µl	15 µl	15 µl
Methylated peptide substrate (200 µM)	5 µl	5 µl	–	5 µl
Test Inhibitor	–	10 µl	–	–
Inhibitor buffer (no inhibitor)	10 µl	–	10 µl	10 µl
1x LSD1 assay buffer 1	–	–	5 µl	20 µl
LSD1 (10–15 ng/µl)	20 µl	20 µl	20 µl	–
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

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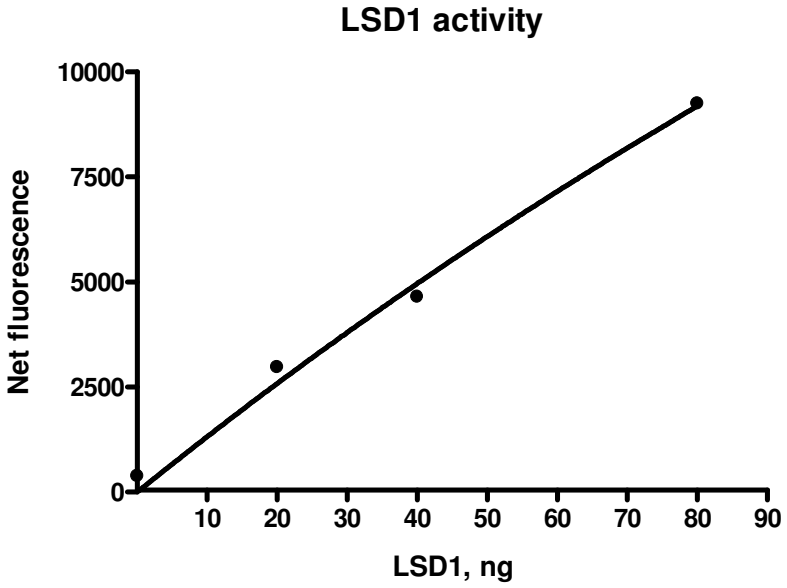

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