LSD1 Inhibitor Screening Assay Kit

Item No. 700120

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -80°C kit. After opening kit, store individual components as stated below.

Item Number	Item	Quantity	Storage
700121	LSD1 Assay Buffer (10X)	1 vial	-20°C
700122	LSD1 (human recombinant) Assay Reagent	2 vials	-80°C
700123	LSD1 Assay Fluorometric Substrate	3 vials	-20°C
700124	LSD1 Assay Horseradish Peroxidase	2 vials	-20°C
700125	LSD1 Assay Peptide	2 vials	-20°C
700001	DMSO Assay Reagent	1 vial	RT
400017	96-Well Plate (black)	1 plate	RT
400012	96-Well Plate Cover	1 cover	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as described in the **Materials Supplied** section, on page 3, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A fluorometer with the capacity to measure fluorescence using an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm
2. Adjustable pipettes and a repeating pipettor
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable

INTRODUCTION

Background

Histones are evolutionarily conserved proteins that are the building blocks of the nucleoprotein chromatin structure that packages DNA within the eukaryotic nucleus. Chromatin contains individual nucleosomal core particles with eight core histone proteins, two copies each of histones H3, H4, H2B, and H2A, with 146 bp of DNA wrapped around each core.[1] The histone amino termini extend from the core where they can be modified post-translationally by acetylation, phosphorylation, ubiquitination, and methylation, which can affect their charge and function. These post-translational modifications affect key DNA regulatory processes such as replication, repair, and transcriptional activation and repression.[1] Dysregulation of histone acetylation and methylation leads to the silencing of tumor suppressor genes and contributes to cancer progression. Inhibitors of enzymes that catalyze the addition and removal of these epigenetic marks thus have therapeutic potential for treating cancer and represent a very active research area in drug development.

Lysine-specific demethylase 1 (LSD1, also identified as KDM1, p110b, BHC110, NPAO, AOF2) belongs to the family of flavin adenine dinucleotide (FAD)-dependent amine oxidases that include monamine oxidases (MAOs) and polyamine oxidase (PAO).[2] LSD1 is a component of several histone deacetylase co-repressor complexes, including histone deacetylases 1/2, CtBP, and the neuronal CoREST complexes.[3] LSD1, with the help of its cofactor CoREST, specifically demethylates mono- and dimethylated histone H3 lysine 4 (H3-K4), resulting in transcriptional repression.[4] In addition to demethylating histones, it has recently been found that LSD1 controls the tumor suppressor activity of p53 by demethylating a specific p53 lysine residue (LYS370).[5] This activity does not seem to require CoREST and p53 demethylation of LYS370 prevents p53 interaction with its co-activator 53BP1 to induce apoptosis.[5-8] The development of LSD1 inhibitors is an important tool for studying the functions of p53 and LSD1 in cell cycle progression.

About This Assay

Cayman's LSD1 Inhibitor Screening Assay Kit provides a convenient fluorescence-based method for screening LSD1-specific inhibitors. The assay is based on the multistep enzymatic reaction in which LSD1 first produces H_2O_2 during the demethylation of lysine 4 on a peptide corresponding to the first 21 amino acids of the N-terminal tail of histone H3. In the presence of horseradish peroxidase (HRP), H_2O_2 reacts with ADHP (10-acetyl-3,7-dihydroxyphenoxazine) to produce the highly fluorescent compound resorufin. Resorufin fluorescence can be easily analyzed with an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm (see Figure 1).

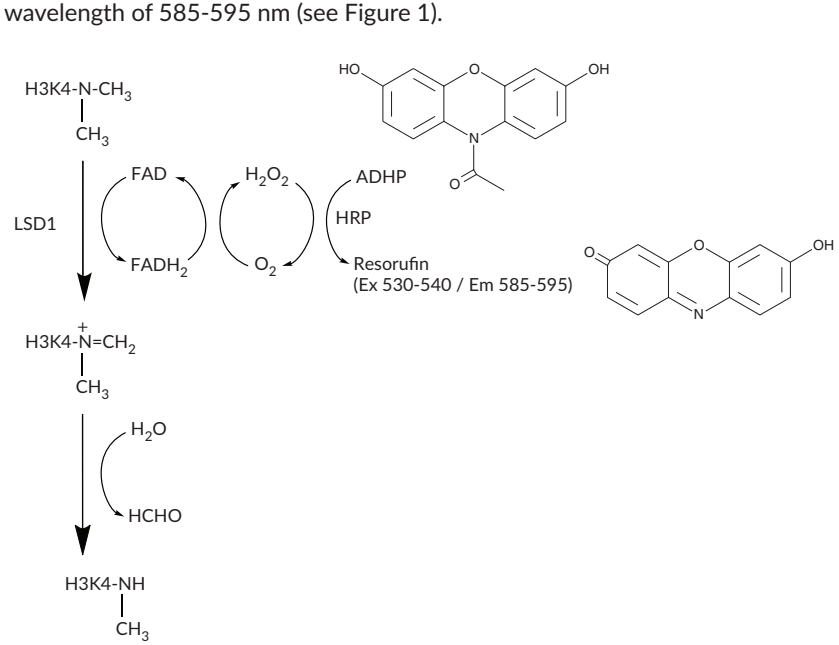

Figure 1. Assay scheme

PRE-ASSAY PREPARATION

Reagent Preparation

1. **LSD1 Assay Buffer (10X) - (Item No. 700121)**

   Dilute 3 ml of Assay Buffer concentrate with 27 ml of HPLC-grade water. This final Buffer (50 mM Hepes, pH 7.5) should be used in the assay and for diluting reagents. When stored at 4°C, this diluted buffer is stable for at least six months.

2. **LSD1 (human recombinant) Assay Reagent - (Item No. 700122)**

   Each vial contains 1 ml of human recombinant LSD1. Thaw the enzyme on ice. The enzyme is ready to use in the assay. One vial of enzyme is enough LSD1 to assay 50 wells. Use the additional vial if utilizing the entire plate.

3. **LSD1 Assay Fluorometric Substrate - (Item No. 700123)**

   The vials contain a clear lyophilized powder of ADHP (10-acetyl-3,7-dihydroxyphenoxazine). Immediately prior to use, dissolve the contents of one vial with 100 µl DMSO Assay Reagent (Item No. 700001) and then add 400 µl of diluted Assay Buffer. The reconstituted substrate is stable for 30 minutes. After 30 minutes, increased background fluorescence will occur.

4. **LSD1 Assay Horseradish Peroxidase - (Item No. 700124)**

   The vials contain a lyophilized powder of horseradish peroxidase (HRP). Dissolve the contents of one vial with 1 ml of diluted Assay Buffer. The reconstituted enzyme is stable for two hours. One vial is enough enzyme to assay 50 wells. If additional wells are being utilized, then reconstitute the other vial.

5. **LSD1 Assay Peptide - (Item No. 700125)**

   The vials contain 1 ml of peptide corresponding to the first 21 amino acids of the N-terminal tail of histone H3, with a dimethylated lysine at residue 4 (ARTK(diMe)QTARKSTGGKAPRKQLA). It is ready to use in the assay. The addition of 20 µl to the assay yields a final concentration of 100 µM. If a lower concentration of peptide is desired, dilute with Assay Buffer. One vial is enough peptide to assay 50 wells. Use the additional vial if utilizing the entire plate.

6. **DMSO Assay Reagent - (Item No. 700001)**

   The vial contains 1 ml of dimethylsulfoxide (DMSO). The reagent is ready to use as supplied.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. However, it is necessary to have three wells designated as 100% initial activity and three wells designated as background wells. We suggest that each inhibitor sample be assayed in triplicate and that you record the contents of each well on the template sheet provided on page 17. A typical layout of samples and compounds to be measured in triplicate is shown below in Figure 2.

	1	2	3	4	5	6	7	8	9	10	11	12
A	BW	BW	BW	7	7	7	15	15	15	23	23	23
B	A	A	A	8	8	8	16	16	16	24	24	24
C	1	1	1	9	9	9	17	17	17	25	25	25
D	2	2	2	10	10	10	18	18	18	26	26	26
E	3	3	3	11	11	11	19	19	19	27	27	27
F	4	4	4	12	12	12	20	20	20	28	28	28
G	5	5	5	13	13	13	21	21	21	29	29	29
H	6	6	6	14	14	14	22	22	22	30	30	30

BW - Background Wells
A - 100% Initial Activity Wells
1-30 - Inhibitor Wells

Figure 2. Sample plate format

Pipetting Hints

- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 200 µl in all the wells.
- Use the diluted Assay Buffer in the assay.
- It is not necessary to use all the wells on the plate at one time.
- If the appropriate inhibitor concentration is not known, it may be necessary to assay at several concentrations.
- We recommend assaying samples in triplicate, but it is the user's discretion to do so.
- Thirty inhibitor samples can be assayed in triplicate or forty-six in duplicate.
- The assay temperature is 37°C.
- Monitor the fluorescence with an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm.

Performing the Assay

1. **100% Initial Activity Wells** - add 120 µl of Assay Buffer, 20 µl of LSD1, 20 µl of HRP, 10 µl of Fluorometric Substrate, and 10 µl of solvent (the same solvent used to dissolve the inhibitor) to three wells.
2. **Background Wells** - add 140 µl of Assay Buffer, 20 µl of LSD1, 20 µl of HRP, 10 µl of Fluorometric Substrate, and 10 µl of solvent (the same solvent used to dissolve the inhibitor) to three wells.
3. **Inhibitor Wells** - add 120 µl of Assay Buffer, 20 µl of LSD1, 20 µl of HRP, 10 µl of Fluorometric Substrate, and 10 µl of inhibitor* to three wells.
4. Initiate the reactions by adding 20 µl of peptide to all the wells being used, except the background wells.
5. Cover the plate with the plate cover and incubate for 30 minutes at 37°C.
6. Remove the plate cover and read the plate using an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm.
7. It may be necessary to adjust the gain setting on the instrument to allow for the measurement of all the samples.

*Inhibitors can be dissolved in Assay Buffer, ethanol, methanol, or DMSO and should be added to the assay in a final volume of 10 µl. In the event that the appropriate concentration of inhibitor needed for LSD1 inhibition is completely unknown, we recommend that several concentrations of the inhibitor be assayed.

ANALYSIS

Calculations

1. Determine the average fluorescence of each sample.
2. Subtract the fluorescence of the background wells from the fluorescence of the 100% initial activity and the inhibitor wells.
3. Determine the percent inhibition for each sample. To do this, subtract each inhibitor sample value from the 100% initial activity sample value. Divide the result by the 100% initial activity value and then multiply by 100 to give the percent inhibition.
4. Either graph the Percent Inhibition or Percent Initial Activity as a function of the inhibitor concentration to determine the IC_{50} value (concentration at which there was 50% inhibition). An example of LSD1 inhibition by *trans*-2-phenylcyclopropylamine (2-PCPA, Item No. 10010494), is shown in Figure 3 on page 14.[9,10]

$$\%\text{Inhibition} = \left[\frac{(\text{Initial Activity} - \text{Sample})}{\text{Initial Activity}}\right] \times 100$$

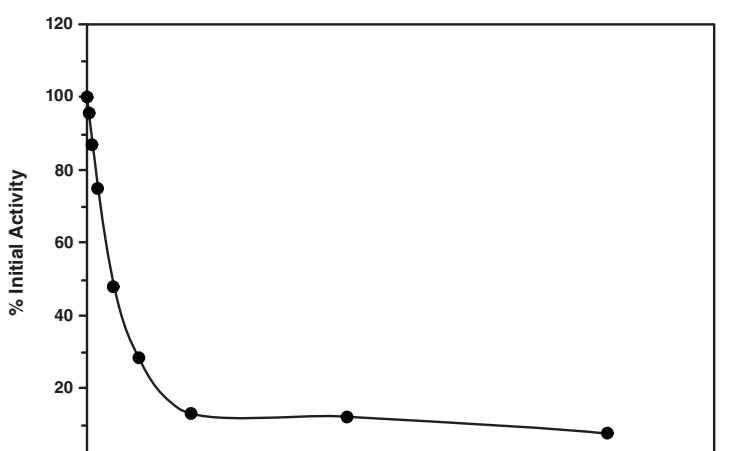

Figure 3. Inhibition of LSD1 by 2-PCPA (IC_{50} = 22 µM)

Performance Characteristics

Precision:

When a series of sixteen LSD1 measurements were performed on the same day, the intra-assay coefficient of variation was 2.9%. When a series of sixteen LSD1 measurements were performed on five different days under the same experimental conditions, the inter-assay coefficient of variation was 3.1%.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No fluorescence detected above background in any of the wells	A. Either substrate or LSD1 was not added to the wells B. The inhibitor concentration was too high	A. Make sure to add all the components to the wells and re-assay B. Reduce the inhibitor concentration and re-assay
The fluorometer exhibited 'MAX' values for the wells	The GAIN setting is too high	Reduce the GAIN. Make sure bandwidths are set at 10 nm and re-read
No inhibition seen with compound	A. The compound concentration is not high enough B. The compound is not an inhibitor of the enzyme	Increase the compound concentration and re-assay

References

1. Strahl, B.D. and Allis, D. The language of covalent histone modifications. *Nature* **403**, 41-45 (2000).
2. Shi, Y., Lan, F., Matson, C., *et al.* Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* **119**, 941-953 (2004).
3. Shi, Y-J., Matson, C., Lan, F., *et al.* Regulation of LSD1 histone demethylase activity by its associated factors. *Molecular Cell* **19**, 864 (2005).
4. Forneris, F., Binda, C., Vanoni, M.A., *et al.* Human histone demethylase LSD1 reads the histone code. *J. Biol. Chem.* **280(50)**, 41360-41365 (2005).
5. Huang, J., Sengupta, R., Espejo, A.B., *et al.* p53 is regulated by the lysine demethylase LSD1. *Nature* **449**, 105-108 (2007).
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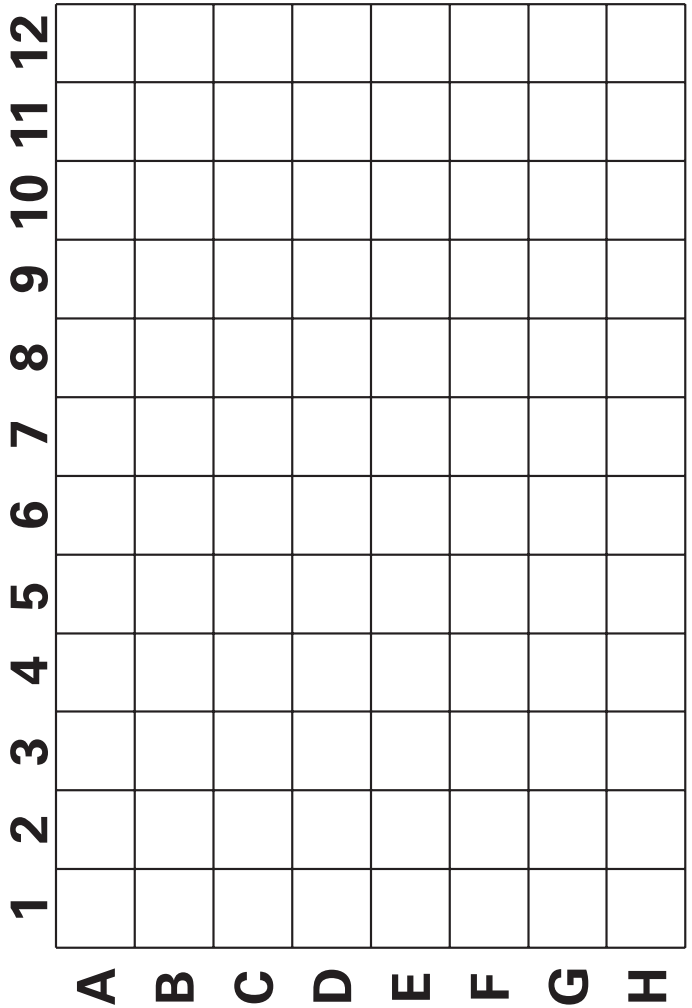
7. Tsai, W-W., Nguyen, T.T., Shi, Y., *et al.* p53-targeted LSD1 functions in repression of chromatin structure and transcription *in vivo*. *Mol. Cell. Biol.* **28(17)**, 5139-5146 (2008).
8. Scoumanne, A., Chen, X. Protein methylation: A new mechanism of p53 tumor suppressor regulation. *Histol Histopathol* **23**, 1143-1149 (2008).
9. Schmidt, D.M.Z. and McCafferty, D.G. *trans*-2-Phenylcyclopropylamine is a mechanism-based inactivator of the histone demethylase LSD1. *Biochemistry* **46**, 4408-4416 (2007).
10. Gooden, D.M., Schmidt, D.M.Z., Pollock, J.A., *et al.* Facile synthesis of substituted *trans*-2-arylcyclopropylamine inhibitors of the human histone demethylase LSD1 and monoamine oxidases A and B. *Bioorg Medicinal Chem Letters* **18**, 3047-3051 (2008).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.