

# Histone Demethylase Assay (Fluorescent)

Catalog No. 53200

(version B1)

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## Overview

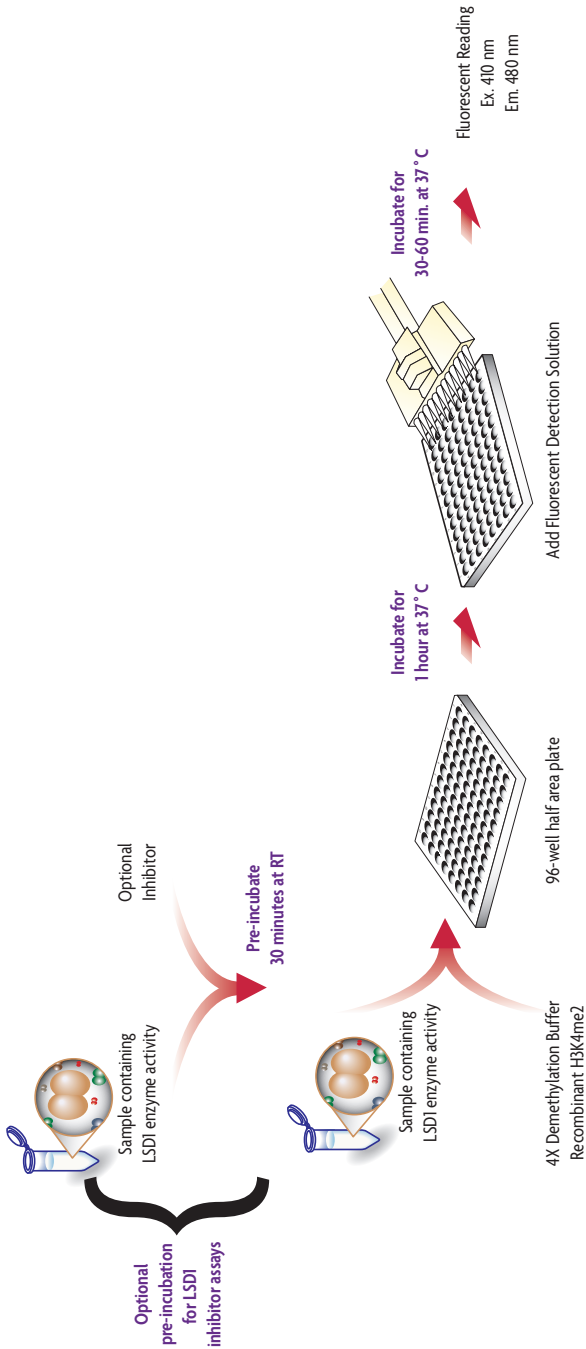
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The Histone Demethylase Assay (Fluorescent) provides a simple solution for screening activity of purified or recombinant lysine specific demethylase (LSD1) enzymes. The assay is designed to detect the formaldehyde released from the reaction of lysine specific demethylase 1 (LSD1) with a methylated substrate. The Recombinant Histone H3K4me2 protein used in the Histone Demethylase Assay mimics a native histone substrate, allowing for results that more closely resemble *in vivo* conditions. As the LSD1 enzyme demethylates the histone substrate, formaldehyde is released as a by-product. The lysine specific demethylase enzymes are capable of demethylating substrates that are either monomethylated or dimethylated, enabling the production of up to two formaldehyde molecules per substrate. The Detection Reagent reacts with each formaldehyde molecule to generate a fluorescent signal equivalent to the overall production of formaldehyde. The Histone Demethylase Assay Kit can be used to analyze the overall histone conversion efficiency of an LSD1 sample, or to screen compounds for changes in histone demethylation activity.

The Histone Demethylase Assay contains a Recombinant Histone H3K4me2 substrate, optimized buffers to enhance enzymatic activity and 96-well black half area plates to perform the reactions. For added convenience, a Demethylation Standard and a small amount of LSD1 enzyme are included as positive controls. The fluorescent signal released upon interaction of the Detecting Reagent with the formaldehyde by-product can be measured using a fluorescent microplate reader with an excitation wavelength of  $410 \pm 20$  nm and an emission wavelength of  $480 \pm 25$  nm.

<b>product</b>	<b>format</b>	<b>catalog no.</b>
Histone Demethylase Assay (Fluorescent)	48 rxns	53200

# Flow Chart of Process



# Introduction

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## Histone Demethylase Enzymes

The basic structural unit of chromatin is the nucleosome, which consists of 146 base pairs (bp) of DNA wrapped around a histone octamer. The histone octamer consists of two copies each of the core histones H2A-H2B dimers and a tetramer of H3-H4. A linker histone, histone H1, binds chromatin outside the nucleosome unit to regulate chromatin structure.

Histone modifications such as phosphorylation, acetylation and methylation at specific amino acid residues on the histone tails that extend beyond the core nucleosome have been found to influence and regulate transcription, chromosome packaging and DNA damage repair. While histone acetylation occurs only on lysine (K) residues, histone methylation occurs at both lysine (K) and arginine (R) residues on the N-terminal histone tails. Histone lysine methylation is of particular interest given its association with transcriptional activation and repression, DNA damage response and X chromosome inactivation<sup>1,2,3,4</sup>.

Histone methyltransferases (HMTs) and histone demethylases (HDMs) regulate histone methylation. There are currently two classes of histone demethylase enzymes: the lysine specific demethylase 1 (LSD1) class and the Jumonji (JmjC)-domain-containing class. Each class of histone demethylase uses a different reaction mechanism, which leads to different substrate specificity and requires the use of different co-factors for enzymatic activity.

The lysine specific demethylase 1 (LSD1) class, also known as KDM1 for lysine (K) demethylase 1, uses a flavin adenine dinucleotide (FAD)-dependent amine oxidase domain for its demethylase activity. FAD is used as a cofactor to catalyze an amine oxidation that results in the release of a formaldehyde molecule. LSD1 can use either di- or mono-methylated H3K4 as a substrate. If a dimethyl H3K4 substrate is used, the demethylation reaction will produce a monomethyl H3K4 substrate that is then capable of undergoing a subsequent reaction to become unmethylated. A formaldehyde molecule will be released at each step<sup>5</sup>.

The Jumonji (JmjC)-domain-containing histone demethylases requires multiple co-factors, such as Fe(II), O<sub>2</sub> and  $\alpha$ -ketoglutarate, to hydroxylate the methyl group and release formaldehyde. The JmjC domain is capable of demethylating mono-, di- and tri-methylated substrates<sup>5</sup>, but are not suitable for use in the Histone Demethylase Assay (Fluorescent).

## Histone Demethylase Assay (Fluorescent)

The Histone Demethylase Assay was designed to detect the formaldehyde released from the reaction of the lysine specific demethylase (LSD1) with a methylated substrate, H3K4me2. As the LSD1 enzyme demethylates the histone substrate, formaldehyde is released as a by-product. The included Detecting Reagent is able to react with the formaldehyde to generate a fluorescent signal that can be measured using a fluorescent microplate reader with an excitation wavelength of 410 $\pm$ 20 nm and an emission wavelength of 480 $\pm$ 25 nm.

## Kit Performance and Benefits

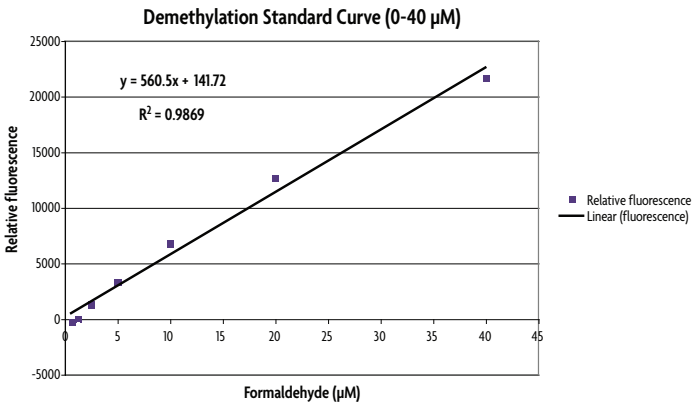
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The Histone Demethylase Assay (Fluorescent) can be used to analyze the overall histone conversion efficiency as a dimethyl histone substrate is converted to either a monomethyl or unmethylated state. The assay can also be used to screen compounds for changes in histone demethylation activity.

**Range of detection:** The assay can detect activity from recombinant proteins or purified enzymes. To determine the optimal conditions for each sample, it is recommended initially to prepare a range of concentrations to determine the level of enzymatic activity for each sample. We suggest a minimum of 100 ng LSD1 enzyme as a starting point.

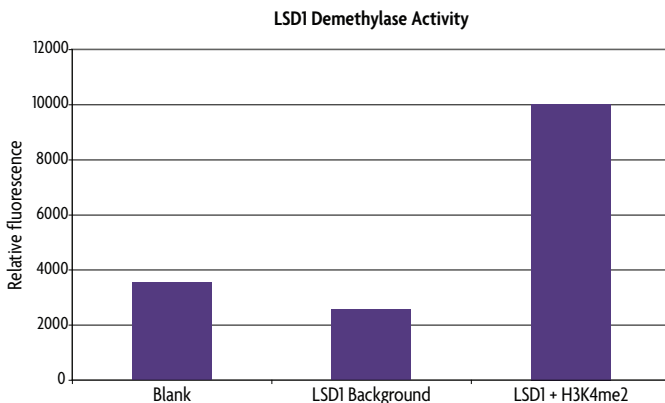
**Assay time:** 2.5 hours.

### Histone Demethylase Assay (Fluorescent)



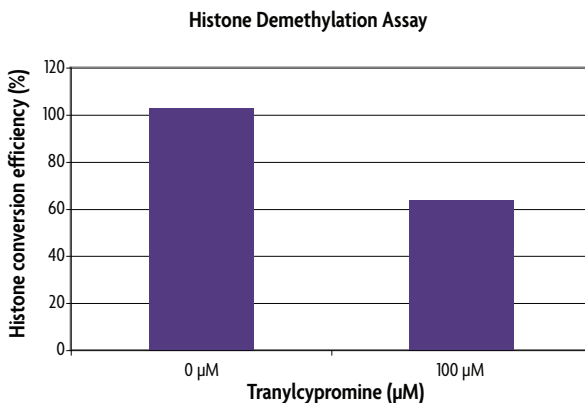
#### Demethylation standard curve showing fluorescence generated from the formaldehyde standard.

The Histone Demethylase Assay was used to generate a formaldehyde standard curve. The Demethylation Standard was assayed from 0-40  $\mu\text{M}$  formaldehyde. The enzymatic reaction was incubated at 37°C for one hour, followed by a one hour incubation with the detection solution before the fluorescent intensity was measured with an excitation wavelength of 410 nm and an emission wavelength of 480 nm. Results were plotted as a linear regression. Data shown are the results from samples assayed in duplicate. These results are provided for demonstration only.



#### Fluorescence detection of LSD1 demethylase activity.

The Histone Demethylase Assay was used to assay activity of the LSD1 positive control. One  $\mu\text{g}$  of LSD1 was tested in the absence or presence of the recombinant H3K4me2 histone substrate. The enzymatic reaction was incubated at  $37^\circ\text{C}$  for one hour, followed by a one hour incubation with the detection solution before the fluorescent intensity was measured with an excitation wavelength of 410 nm and an emission wavelength of 480 nm. Data shown are the results from samples assayed in duplicate. These results are provided for demonstration only.



#### Histone Demethylase Assay to evaluate LSD1 inhibition by Tranylcypromine.

The Histone Demethylase Assay was used to assay activity of the LSD1 positive control in the absence or presence of the irreversible LSD1 inhibitor, Tranylcypromine.  $100 \mu\text{M}$  Tranylcypromine was added to  $1 \mu\text{g}$  LSD1 and pre-incubated for 30 minutes at room temperature. Following the pre-incubation, the enzyme and inhibitor combination was added to the reaction plate containing the H3K4me2 histone substrate. The enzymatic reaction was incubated at  $37^\circ\text{C}$  for one hour, followed by a one hour incubation with the detection solution before the fluorescent intensity was measured with an excitation wavelength of 410 nm and an emission wavelength of 480 nm. A demethylation standard curve was used to determine the amount of formaldehyde released, which was used to calculate the histone conversion efficiency. Data shown are the results from samples assayed in duplicate. These results are provided for demonstration only.



## Kit Components and Storage

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Histone Demethylase Assay Kits are for research use only. Not for use in diagnostic procedures. All components are guaranteed stable for 6 months from date of receipt when stored properly.

Reagents	Quantity	Storage
LSD1 enzyme (control)	5 µg	-80°C
Recombinant Histone H3K4me2 (substrate)	1 vial	-80°C
4X Demethylation Buffer	5 ml	-20°C
Demethylation Standard (1 M)	75 µl	4°C
Detection Buffer AM1	5 ml	4°C
Detection Reagent AM1	75 µl	4°C
96-well black half area assay plate	2	RT
Plate sealer	2	RT

### Additional materials required

- Histone demethylase enzyme samples (recombinant or purified)
- Multi-channel pipettor
- Conical and microcentrifuge tubes for reagent dilution
- Incubator set at 37°C
- Fluorescent microplate reader with filters capable of reading an excitation wavelength of  $410\pm 20$  nm and an emission wavelength of  $480\pm 25$  nm

### Preparing lysine specific histone demethylase (LSD1) samples

Histone demethylase samples can be prepared using several techniques:

1. Recombinant enzyme sources can be used directly in the Histone Demethylase Assay (Fluorescent).
2. Lysine specific histone demethylase proteins can be immunoprecipitated from whole-cell or nuclear extracts. It is important to find an antibody that will bind to the specific demethylase but does not interfere with the activity of the histone demethylase enzyme.

## Protocols

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### Buffer Preparation and Recommendations

#### 4X Demethylation Buffer

The Demethylation Buffer can be used at the 4X concentration for setting up the individual enzymatic reactions. Prepare 1X Demethylation Buffer for the resuspension of the histone substrate and the dilution of the LSD1 positive control enzyme and test samples.

For the Recombinant Histone H3K4me2: In a microcentrifuge tube, add 130  $\mu$ l of 4X Demethylation to 390  $\mu$ l sterile deionized water to create 520  $\mu$ l of 1X Demethylation Buffer.

For LSD1 control enzyme or test samples: Prepare the amount of 1X Demethylation Buffer required to dilute positive control and test samples. The 4X Demethylation Buffer should be diluted 1:4 in sterile deionized water.

#### Recombinant Histone H3K4me2

The Recombinant Histone H3K4me2 protein is provided lyophilized. Resuspend the recombinant histone by adding 500  $\mu$ l 1X Demethylation Buffer to the vial. Resuspend by pipetting up and down. Keep the vial on ice and vortex occasionally over 20 minutes to ensure full resuspension of the recombinant protein. Vortex the vial and then centrifuge to collect the contents at the bottom before using the histone substrate. Use 10  $\mu$ l per reaction (3.268  $\mu$ M). Enough recombinant protein is provided to serve as the histone demethylase substrate for all 48 reactions.

**Note:** It is important to avoid repeated freeze-thaw cycles of the substrate. We recommend making 50  $\mu$ l aliquots of any unused substrate and storing at  $-80^{\circ}\text{C}$ .

#### Demethylation Standard

The Demethylation Standard is a 1 M formaldehyde stock solution. To prepare the standard, make a 1:1000 dilution by adding 10  $\mu$ l Demethylation Standard into 10 ml sterile deionized water to produce a 1 mM working solution. Follow the instructions for preparing the Demethylation Standard curve on page 10.

**WARNING:** Formaldehyde is a toxic and combustible liquid. Wear personal protective equipment when handling, *i.e.* safety glasses, gloves and labcoat. Formaldehyde should only be used in well-ventilated areas.

#### LSD1 enzyme

Centrifuge the LSD1 enzyme vial prior to opening to ensure complete recovery of the contents. The enzyme is provided at a concentration of 0.5 mg/ml. Dilute the enzyme 1:5 in 1X Demethylation Buffer. LSD1 enzyme should be diluted fresh for each experiment. For each positive control reaction add 2  $\mu$ l LSD1 enzyme to 8  $\mu$ l 1X Demethylation Buffer. This makes a 100 ng/ $\mu$ l working stock. Keep on ice until ready to use. Enough LSD1 enzyme is provided for 5 positive control reactions.

**Note:** Once LSD1 enzyme is diluted, do not reuse. Discard diluted enzyme.

## Samples

It is recommended initially to use a range of sample concentrations (e.g. 100 ng, 1 µg) in order to identify the optimal enzymatic conditions for your sample. Preparing a range of sample concentrations will help to ensure the enzymatic reaction is within the detectable range. Samples should be diluted in 1X Demethylation Buffer and used in a final volume of 10 µl per reaction. We suggest a minimum of 100 ng LSD1 enzyme as a starting point

Once sample demethylase activity has been determined within the assay, perform the rest of the assays using sample concentrations that fall within the linear range of the Demethylation Standard curve.

## Detection Solution

It is recommended to prepare the Detection Solution at the end of the enzymatic reaction incubation. Detection Solution should be prepared immediately before adding to the plate. To prepare detection solution, add 5 µl of Detection Reagent AMI per 1 ml of Detection Buffer AMI. Vortex to mix. Use 25 µl per reaction. Discard any unused Detection Solution.

## Assay Protocol

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### Read the entire protocol before use.

Determine the appropriate number of microwells required for testing samples, controls, demethylation standards and blanks in duplicate. It is recommended to perform all the reactions on the same plate. Following the incubation with Detection Solution, reactions will be transferred to a new plate to read the fluorescence. Cover unused wells with a portion of the plate sealer while you perform the assay.

### Histone Demethylase Assay (Fluorescent) Protocol

Prepare the Demethylation Standard, Recombinant Histone H3K4me2 substrate, LSD1 positive control and samples as described above in the section Buffer Preparation and Recommendations. Add the demethylation standard, test samples, positive control and blank reactions to the same plate in order to synchronize the incubation steps. Multi-channel pipettes and reservoirs can be used for addition of reagents.

### Optional: Testing LSD1 inhibitors

The Histone Demethylase Assay can be used to screen for LSD1 inhibitors. In this case, a pre-incubation step allowing for the association of the enzyme and the inhibitor is recommended prior to addition to the histone substrate.

1. Prepare samples and positive controls as described in the section Buffer Preparation and Recommendations. Prepare the inhibitor, such that the volume of inhibitor added will not cause the final enzymatic reaction volume to exceed 100  $\mu$ l (as described on page 11).
2. In a microcentrifuge tube, combine the enzyme and the inhibitor. Incubate for at least 30 minutes at room temperature. Do not continue on to Step 1 of the protocol until the pre-incubation with the inhibitor is nearly completed.

## Step 1: Demethylation Standard Curve Preparation

Use this plate set-up example to prepare a formaldehyde standard curve at a range of 0-40  $\mu\text{M}$  for the Histone Demethylase Assay (Fluorescent) in duplicate. The standard curve should be prepared on the same plate as the test and control samples.

		1	2	3	4	5	6	7	8	9	10	11	12
75 $\mu\text{l}$	A	40 $\mu\text{M}$	40 $\mu\text{M}$	–	–	–	–	–	–	–	–	–	–
75 $\mu\text{l}$	B	20 $\mu\text{M}$	20 $\mu\text{M}$	–	–	–	–	–	–	–	–	–	–
75 $\mu\text{l}$	C	10 $\mu\text{M}$	10 $\mu\text{M}$	–	–	–	–	–	–	–	–	–	–
75 $\mu\text{l}$	D	5 $\mu\text{M}$	5 $\mu\text{M}$	–	–	–	–	–	–	–	–	–	–
75 $\mu\text{l}$	E	2.5 $\mu\text{M}$	2.5 $\mu\text{M}$	–	–	–	–	–	–	–	–	–	–
75 $\mu\text{l}$	F	1.25 $\mu\text{M}$	1.25 $\mu\text{M}$	–	–	–	–	–	–	–	–	–	–
75 $\mu\text{l}$	G	0.63 $\mu\text{M}$	0.63 $\mu\text{M}$	–	–	–	–	–	–	–	–	–	–
	H	0 $\mu\text{M}$	0 $\mu\text{M}$	–	–	–	–	–	–	–	–	–	–

1. Dilute the Demethylation Standard as described in the Buffer Preparation and Recommendation section on page 7.
2. Pipette 144  $\mu\text{l}$  of sterile DI water to wells A1 and A2.
3. Pipette 75  $\mu\text{l}$  of sterile DI water to wells B1 through H2.
4. Add 6  $\mu\text{l}$  of the 1 mM Demethylation Standard to wells A1 and A2. With a pipette set at 100  $\mu\text{l}$ , pipet up and down several times to mix the solution. The final concentration of formaldehyde is 40  $\mu\text{M}$ .
5. Perform a serial two-fold dilution of the standard by transferring 75  $\mu\text{l}$  of the 40  $\mu\text{M}$  formaldehyde solution in row A to the wells in row B.
6. Mix the contents of row B by pipetting up and down 3-5 times. Do not change pipette tips between well transfers.
7. Transfer 75  $\mu\text{l}$  of the contents of row B to row C and mix, as previously described.
8. Continue this process until row G is reached.
9. When row G is reached, discard 75  $\mu\text{l}$  of the well contents so that the final volume is 75  $\mu\text{l}$ .
10. Row H will serve as the blank wells.
11. Add 25  $\mu\text{l}$  of 4X Demethylation Buffer to all wells and pipet to mix. The final volume in each well will be 100  $\mu\text{l}$ .
12. Immediately proceed to Step 2 to prepare sample reactions on the same plate.

## Step 2: Sample Preparation

1. If testing inhibitors, make sure to perform the optional pre-incubation reaction on page 9.
2. Prepare samples and positive controls as described in the section Buffer Preparation and Recommendations. We suggest running a Blank, Sample Background and Sample with Substrate reaction for each sample being tested. The sample background can be used to determine if there is any interference from the sample with the fluorescent readout.

	Demethylation Standard		Enzymatic Reactions									
	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	40 $\mu$ M	40 $\mu$ M	Blank		-	-	-	-	-	-	-	-
<b>B</b>	20 $\mu$ M	20 $\mu$ M	Sample Background		-	-	-	-	-	-	-	-
<b>C</b>	10 $\mu$ M	10 $\mu$ M	Sample + Substrate		-	-	-	-	-	-	-	-
<b>D</b>	5 $\mu$ M	5 $\mu$ M	Positive Control		-	-	-	-	-	-	-	-
<b>E</b>	2.5 $\mu$ M	2.5 $\mu$ M	-	-	-	-	-	-	-	-	-	-
<b>F</b>	1.25 $\mu$ M	1.25 $\mu$ M	-	-	-	-	-	-	-	-	-	-
<b>G</b>	0.63 $\mu$ M	0.63 $\mu$ M	-	-	-	-	-	-	-	-	-	-
<b>H</b>	0 $\mu$ M	0 $\mu$ M	-	-	-	-	-	-	-	-	-	-

3. Using the table below, add the reagents to each well of the plate in the order listed below.

Reagents	Blank	Sample Background	Sample + Substrate	Positive Control
Sterile DI H <sub>2</sub> O	65 $\mu$ l	_____ $\mu$ l	_____ $\mu$ l	55 $\mu$ l
4X Demethylation Buffer	25 $\mu$ l	25 $\mu$ l	25 $\mu$ l	25 $\mu$ l
Recombinant H3K4me2	10 $\mu$ l	None	10 $\mu$ l	10 $\mu$ l
Demethylase Enzyme (with optional inhibitor)	None	_____ $\mu$ l	_____ $\mu$ l	10 $\mu$ l
<b>Total Volume</b>	<b>100 <math>\mu</math>l</b>	<b>100 <math>\mu</math>l</b>	<b>100 <math>\mu</math>l</b>	<b>100 <math>\mu</math>l</b>

4. Immediately proceed to Step 3 for the incubations and fluorescent detection.

### Step 3: Incubations and Fluorescent Detection

1. Cover the plate containing the demethylation standards, positive controls and samples with plate sealer to prevent evaporation. Incubate the plate at 37°C for 1 hour.
2. Prepare Detection Solution as stated in the Buffer Preparation and Recommendation section about 5 minutes before the end of the incubation.
3. Using a multi-channel pipette, add 25  $\mu$ l of the working Detection Solution to all wells. Mix thoroughly. Change tips between each sample.
4. Cover the plate with plate sealer to prevent evaporation. Incubate the plate at 37°C for 30-60 minutes.
5. During the incubation, prepare the fluorescent plate reader. A 20  $\mu$ sec integration time and a gain of 65 is recommended. Alternatively, the gain can be set at optimal.
6. As bubbles may be present in the reaction wells and interfere with the fluorescence of the sample, after the incubation with the Detection Solution transfer 100  $\mu$ l of each demethylation reaction to a new plate. Be careful not to transfer any bubbles.
7. Read fluorescence in a plate reader with excitation wavelength at 410 nm and emission wavelength at 480 nm.

#### Calculation of histone conversion efficiency

1. Calculate the average value for each duplicate data point.
2. Subtract the fluorescent intensity of the blank values (H1 and H2 of the standard curve) from the wells containing the formaldehyde standards. Also subtract the blank values (A3 and A4) from the test samples and positive control. These values are relative fluorescent intensity.
3. To generate a standard curve, plot the relative fluorescent intensity per point on the y-axis versus the concentration of formaldehyde in the Demethylation Standard on the x-axis. Generate a linear trendline for the plotted standard. The standard curve should have an  $R^2$  value greater than 0.95 to be used for extrapolation of data. See example graph on page 4.
4. From the curve plotted in Step 3, extrapolate the concentration of formaldehyde released from the samples based on the linear regression equation generated.
5. To determine the histone conversion efficiency, divide the extrapolated formaldehyde concentration in  $\mu$ M units by the histone molar concentration of the H3K4me2 substrate used in each reaction (10  $\mu$ l Recombinant Histone H3K4me2 = 3.268  $\mu$ M). Multiply by 100 to obtain a percentage.

The conversion efficiency calculated represents the overall conversion of a methylated substrate into a formaldehyde by-product. Since the H3K4me2 histone substrate is capable of releasing up to two formaldehyde molecules each, histone conversion efficiencies between 100-200% are expected for active LSD1 samples. Individual conversion efficiencies between the dimethylated histone state and monomethylated histone state cannot be individually determined in this assay.

## References

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1. Shi, Y. *et al* (2004) *Cell* 119: 941-953.
2. Zhang, Y. and Reinberg, D. (2001) *Genes Dev.* 15: 2343-2360.
3. Margueron, R. *et al* (2005) *Genet. Dev.* 15: 163-176.
4. Martin, C. and Zhang, Y. (2005) *Nat. Rev. Mol. Cell Biol.* 6: 838-849.
5. Nottke, A. *et al* (2009) *Development* 136: 879-889.

## Appendix

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### Section A: Troubleshooting Guide

Problem/question	Possible cause	Recommendation
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in all wells in the correct order.
	Not using a lysine specific demethylase (LSDI) sample	The Histone Demethylase Assay is optimized for use with the lysine specific histone demethylase, LSDI. The assay is not designed to work with the JmjC class of histone demethylases.
	Weak enzymatic activity	Try a range of sample concentrations in order to determine the optimal enzymatic conditions for the test sample. For histone demethylase enzymes with low enzymatic activity, longer enzymatic reaction incubation times (e.g. 3-5 hours) can be performed.
	Enzyme inhibitor present	Prepare histone demethylase samples with clean reagents that have not been in contact with inhibitors. If testing an inhibitor, make sure to change tips between samples.
	Plate reader settings not optimal	Verify the wavelength and filter settings in the plate reader. Use a 20 $\mu$ sec integration time and select an optimal gain setting, or use a gain of 65.
	Incorrect assay temperature	Enzymatic and detecting reactions should be performed at 37C.
High fluorescent signal in sample background wells	Concentration of histone demethylase enzyme is too high	It is recommended initially to test a range of sample concentration to determine the optimal amount for use in the assay. The sample values should fall within the linear range of the demethylation standard curve.



## Section B. Related Products

Histone ELISAs	Format	Catalog No.
Histone H3 monomethyl Lys4 ELISA	1 x 96 rxns	53101
Histone H3 dimethyl Lys4 ELISA	1 x 96 rxns	53112
Histone H3 trimethyl Lys4 ELISA	1 x 96 rxns	53113
Histone H3 dimethyl Lys9 ELISA	1 x 96 rxns	53108
Histone H3 trimethyl Lys9 ELISA	1 x 96 rxns	53109
Histone H3 monomethyl Lys27 ELISA	1 x 96 rxns	53104
Histone H3 trimethyl Lys27 ELISA	1 x 96 rxns	53106
Histone H3 phospho Ser10 ELISA	1 x 96 rxns	53111
Histone H3 phospho Ser28 ELISA	1 x 96 rxns	53100
Total Histone H3 ELISA	1 x 96 rxns	53110

Recombinant Methylated Histones	Format	Catalog No.
Recombinant Histone H2A	50 µg	31251
Recombinant Histone H2B	50 µg	31252
Recombinant Histone H3 (C110A)	50 µg	31207
Recombinant Histone H3 monomethyl Lys4	50 µg	31208
Recombinant Histone H3 dimethyl Lys4	50 µg	31209
Recombinant Histone H3 trimethyl Lys4	50 µg	31210
Recombinant Histone H3 monomethyl Lys9	50 µg	31211
Recombinant Histone H3 dimethyl Lys9	50 µg	31212
Recombinant Histone H3 trimethyl Lys9	50 µg	31213
Recombinant Histone H3 monomethyl Lys27	50 µg	31214
Recombinant Histone H3 dimethyl Lys27	50 µg	31215
Recombinant Histone H3 trimethyl Lys27	50 µg	31216
Recombinant Histone H3 monomethyl Lys36	50 µg	31217
Recombinant Histone H3 dimethyl Lys36	50 µg	31218
Recombinant Histone H3 trimethyl Lys36	50 µg	31219
Recombinant Histone H3 monomethyl Lys79	50 µg	31220
Recombinant Histone H3 dimethyl Lys79	50 µg	31221
Recombinant Histone H3 trimethyl Lys79	50 µg	31222
Recombinant Histone H4	50 µg	31223
Recombinant Histone H4 monomethyl Lys20	50 µg	31224
Recombinant Histone H4 dimethyl Lys20	50 µg	31225
Recombinant Histone H4 trimethyl Lys20	50 µg	31226

Control Acid Extracts	Format	Catalog No.
HeLa acid extract	100 µg	36200
HeLa acid extract (Paclitaxel treated)	100 µg	36201
HeLa acid extract (Sodium Butyrate treated)	100 µg	36202
HeLa acid extract (Etoposide treated)	100 µg	36203
HeLa acid extract (Anacardic acid treated)	100 µg	36204

Histone Purification	Format	Catalog No.
Histone Purification Kit	10 rxns	40025
Histone Purification Mini Kit	20 rxns	40026
HeLa Core Histones	36 µg	53501

<b>Co-Immunoprecipitation</b>	<b>Format</b>	<b>Catalog No.</b>
Universal Magnetic Co-IP Kit	25 rxns	54002
Nuclear Complex Co-IP Kit	50 rxns	54001

<b>SUMOylation</b>	<b>Format</b>	<b>Catalog No.</b>
SUMOlink™ SUMO-1 Kit	20 rxns	40120
SUMOlink™ SUMO-2/3 Kit	20 rxns	40220

<b>ChIP-IT™ Kits</b>	<b>Format</b>	<b>Catalog No.</b>
ChIP-IT™ Express	25 rxns	53008
ChIP-IT™ Express Enzymatic	25 rxns	53009
ChIP-IT™ Express HT	96 rxns	53018
Re-ChIP-IT™	25 rxns	53016
ChIP-IT™	25 rxns	53001
ChIP-IT™ Enzymatic	25 rxns	53006
ChIP-IT™ Shearing Kit	10 rxns	53002
Enzymatic Shearing Kit	10 rxns	53005
ChIP-IT™ Protein G Magnetic Beads	25 rxns	53014
Salmon Sperm DNA/Protein G agarose	25 rxns	53003
ChIP-IT™ Control Kit – Human	5 rxns	53010
ChIP-IT™ Control Kit – Mouse	5 rxns	53011
ChIP-IT™ Control Kit – Rat	5 rxns	53012
Ready-to-ChIP HeLa Chromatin	10 rxns	53015
Ready-to-ChIP Hep G2 Chromatin	10 rxns	53019
Ready-to-ChIP K-562 Chromatin	10 rxns	53020
Ready-to-ChIP NIH/3T3 Chromatin	10 rxns	53021

<b>Fluorescent Dyes</b>	<b>Excitation / Emission</b>	<b>Format</b>	<b>Catalog No.</b>
Chromoem™ 488 Carboxylic Acid	488 nm / 517 nm	1 mg	15510
Chromoem™ 488 NHS-Ester	488 nm / 517 nm	1 mg	15511
Chromoem™ 488 Antibody Labeling Kit	488 nm / 517 nm	1 kit	15090
Chromoem™ 494 Carboxylic Acid	494 nm / 628 nm	1 mg	15110
Chromoem™ 494 NHS-Ester	494 nm / 628 nm	1 mg	15111
Chromoem™ 494 Antibody Labeling Kit	494 nm / 628 nm	1 kit	15091
Chromoem™ 505 Carboxylic Acid	505 nm / 526 nm	1 mg	15610
Chromoem™ 505 NHS-Ester	505 nm / 526 nm	1 mg	15611
Chromoem™ 546 Carboxylic Acid	545 nm / 561 nm	1 mg	15210
Chromoem™ 546 NHS-Ester	545 nm / 561 nm	1 mg	15211
Chromoem™ 546 Antibody Labeling Kit	545 nm / 561 nm	1 kit	15092
Chromoem™ 642 Carboxylic Acid	642 nm / 660 nm	1 mg	15310
Chromoem™ 642 NHS-Ester	642 nm / 660 nm	1 mg	15311
Chromoem™ 642 Antibody Labeling Kit	642 nm / 660 nm	1 kit	15093

<b>Fluorescent Protein Labeling</b>	<b>Format</b>	<b>Catalog No.</b>
LigandLink™ pLL-1 Kit	1 kit	34001
LigandLink™ pLL-1-NFκB p65 Kit	1 kit	34004
LigandLink™ pLL-1-p53 Kit	1 kit	34005
LigandLink™ pLL-1-STAT1 Kit	1 kit	34006
LigandLink™ Fluorescein Label	300 rxns	34101
LigandLink™ Hexachlorofluorescein Label	300 rxns	34104

## Technical Services

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If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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