Histone Demethylase Fluorescent Activity Kit

Catalog Number EIAHDMF (192 tests)

Rev 1.0

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Histone Demethylase Fluorescent Activity Kit is a fluorescent activity assay designed to measure histone demethylase activity in a variety of samples. The fluorescent reaction is initiated with the Formaldehyde Reagent which produces a fluorescent signal (450 nm excitation, 510 nm emission) when added to formaldehyde containing samples.

This assay measures the activity of LSD1 and Jumonji family demethylases. The assay was validated with human histone demethylase, but can be used with samples from other species. Most HDM enzyme reactions should be compatible with the assay. However, because HDMs are slow turnover enzymes the amount of formaldehyde produced is low, and only plate readers capable of measuring low fluorescent signals and having adjustable gain or filter settings may be compatible.

Histone demethylases (HDMs) demethylate drugs and other xenobiotic compounds by catalyzing the site-specific demethylation of methyl-lysine residues in histones. There are two known classes of HDMs: the flavin adenine dinucleotide (FAD)-dependent Lysine Specific Demethylase 1 (LSD1) family and the Fe(II)-dependent Jumonji C (JmjC) family. Although they two families of HDMs employ different cofactors and catalytic mechanisms, both produce formaldehyde as a byproduct of the demethylation reaction.

Contents and storage

Upon receipt, store all kit components **except** Demethylase Cell Lysis Buffer at 2°C to 8°C. Store Demethylase Cell Lysis Buffer at –20°C. Store the Demethylase Cell Lysis Buffer at –20°C or lower after opening.

Components	Quantity
Formaldehyde Standard; 2,000 µM formaldehyde solution in a special stabilizing solution, **keep tightly sealed**	500 µL
LSD1-type Assay Buffer; phosphate buffer containing detergents and stabilizers	60 mL
JMJD2A-type Assay Buffer; HEPES buffer containing stabilizers	60 mL
Black 96-well Half Area Plate	2 plates
Formaldehyde Reagent; contains 0.09% sodium azide as a preservative	5 mL
Demethylase Cell Lysis Buffer; Tris based buffer containing detergents, **store frozen**	100 mL
Plate sealer	2

Materials required but not supplied

- Distilled or deionized water
- Fluorescence microtiter plate reader with software capable of measurement at or near 510 nm, with excitation at 450 nm
- 30°C and 37°C incubator
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- Cofactors, enzyme substrates, inhibitors, and/or activators necessary for demethylase activity

Procedural guidelines

- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
- Formaldehyde is a toxic, volatile, reactive chemical. Use in a well-ventilated laboratory. Dispose of all excess standards and samples in a 10% aqueous solution of sodium bisulfite, or according to the appropriate institutional guidelines.

Sample preparation guidelines

- Prepare cell lysates using the Demethylase Cell Lysis Buffer provided with the kit.
  Cell lysis buffers containing SDS and Triton™ X-100 inhibit the formaldehyde signal reaction and should not be used.
- **Important**: Formaldehyde is a toxic, volatile, reactive chemical. Use in a well-ventilated laboratory. Dispose of all excess standards and samples in a 10% aqueous solution of sodium bisulfite, or according to the appropriate institutional guidelines.

Dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

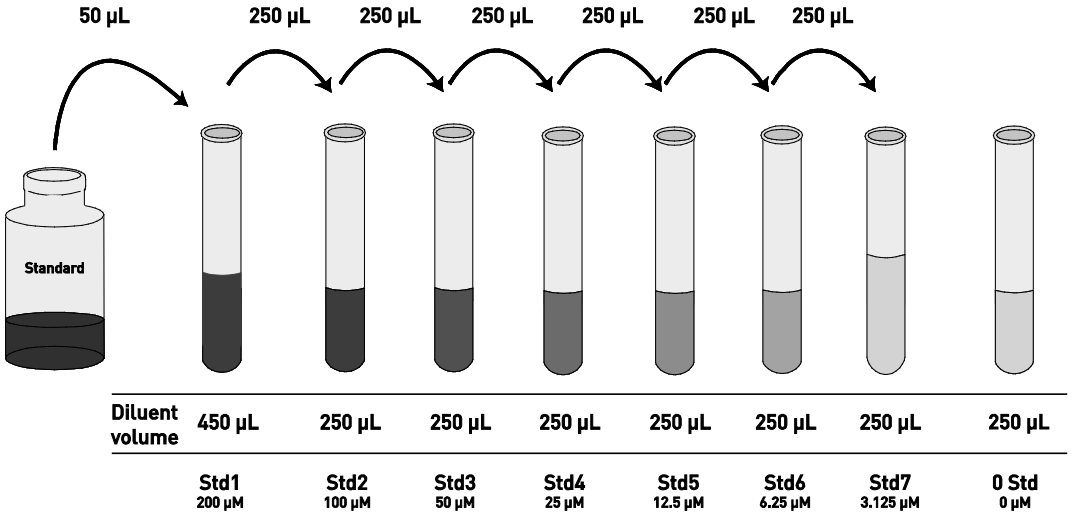
Use all samples within **2 hours** of dilution.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Important: Dilute **standards with the appropriate Assay Buffer containing all necessary cofactors and additives**.

1. Add 50 µL Formadehyde Standard to one tube containing 450 µL Assay Buffer and label as 200 µM formaldehyde.
2. Add 250 µL Assay Buffer to each of 7 tubes labeled as follows: 100, 50, 25, 12.5, 6.25, 3.125, and 0 µM formaldehyde.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. **Use the standards within 2 hours of preparation**.



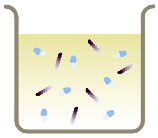
Assay procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 30 minutes.**

IMPORTANT! Perform a standard curve with each assay.

Guidelines for setting up the demethylase reaction

- Demethylase reaction volume should be no more than 100 µL in each well including all cofactors, inhibitors, and activators diluted in the Assay Buffer, or cell lysis buffer for cell lysates.
- Prepare enzyme reaction mix.
  - For a typical LSD1 enzyme reaction, add 50 µL of LSD1 enzyme to 50 µL of a specific 1-21 sequence of Histone H3 with the dimethylated lysine at amino acid 4 (H^3K^4-Me_2) plus all cofactors and inhibitors dissolved in LSD1-type Assay Buffer.
  - For a typical JMJD2A enzyme reaction, add 25 µL of JMJD2A enzyme to 50 µL of a 2 mM ascorbate, 100 µM $FeSO_4$ solution, and 25 µL of a specific 1-24 sequence of Histone H3 with the trimethylated lysine at amino acid 9 (H^3K^9-Me_3) containing 2 mM alpha-ketoglutarate plus all cofactors and inhibitors dissolved in Jumonji-type Assay Buffer.



Add demethylase reaction

a. Set up the demethylase reaction in the appropriate Assay Buffer.
b. Add 100 µL of standards or demethylase reaction mix to each well.
c. Cover the plate with a plate sealer and incubate for 1 hour at 30°C. [1]

Add fluorescent detection reagent

a. Add 25 µL of Formaldehyde Reagent into each well and reseal the plate.
b. Tap the side of the plate to mix.
c. Incubate for 30 minutes at 37°C.

[1] The incubation time used in this protocol was established for the conditions described under "Guidelines for setting up the demethylase reaction." Because conditions may vary, each investigator should determine the optimal time for each application.

Read the plate and generate the standard curve

1. Read the fluorescent emission at 510 nm, with excitation at 450 nm.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background fluorescence may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the activity of unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than that of the highest standard in appropriate Assay Buffer and reanalyze. Multiply the activity by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards.

LSD1 concentration (µM)	Mean FLU
0.128	18,522
0.256	12,410
0.64	6,849
0	1,827

JMJD2A concentration (µM)	Mean FLU
10	17,973
5	10,719
2.5	4,528
0	3,028

Performance characteristics, continued

Interferents (JMJD2A)

The following additives were added to the JMJD2A enzyme reaction with its 1-24 methylated peptide substrate to test for interference with the signal generation.

- The organic solvents methanol, N,N-dimethylformamide and dimethylsulfoxide, each at 5%, had no negative effect on the generation of fluorescence in the presence of JMJD2A.
- 0.005% SDS inhibited the enzymatic generation of formaldehyde by 7.1%, while 0.1% Triton™ X-100 inhibited the signal by 4.2%. Tween™ 20 at ≤0.01% had no effect on signal generation.
- 0.09% sodium azide in the assay buffer reduced the signal by 35.3%, while 0.09% Kathon™ inhibited the signal by 56.9%. Gentamicin at 0.005% had no negative effect on the signal generation.

Interferents (LSD1)

The following additives were added to the LSD1 enzyme reaction with its 1-21 methylated peptide substrate to test for interference with the signal generation.

- The organic solvents methanol, N,N-dimethylformamide and dimethylsulfoxide, each at 5%, had no negative effect on the generation of fluorescence in the presence of 0.256 µM LSD1.
- 0.005% SDS inhibited the enzymatic generation of formaldehyde by 89%, while Triton™ X-100 and Tween™ 20 at ≤0.01% had no effect on signal generation.
- 0.09% sodium azide in the assay buffer reduced the signal by 20%, while 0.09% Kathon™ inhibited the signal by 91.5%. Gentamicin at 0.005% had no negative effect on the signal generation.

Limited product warranty

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