Histone Demethylase Assay (Fluorescent)

screen LSD1 for histone demethylase activity

The fluorescent Histone Demethylase Assay provides a simple solution for screening the activity of purified or recombinant lysine specific demethylase enzymes (LSD1, also known as KDM1). The recombinant histone substrate used in the assay produces results that more closely resemble *in vivo* conditions. As the LSD1 enzyme demethylates the histone substrate, formaldehyde is released as a by-product. The Detection Reagent reacts with formaldehyde molecules to generate a fluorescent signal that is equivalent to the overall production of formaldehyde. The fluorescent signal is then measured, which is used to quantitate the histone demethylase activity.

Measure histone demethylase activity or screen for enzyme inhibition

The Histone Demethylase Assay Kit can be used to analyze the overall histone conversion efficiency of an LSD1 sample, or to screen inhibitor compounds that change histone demethylation activity. The Histone Demethylase Assay contains a Recombinant Histone H3K4me2 substrate, optimized buffers to enhance enzymatic activity and black 96-well black half area microplates to perform the assay (Figure 1). For added convenience, a Demethylation Standard and an aliquot of LSD1 enzyme are included as controls. The fluorescent signal released upon binding of the Detecting Reagent to the formaldehyde by-product can be measured using a fluorescent microplate reader with an excitation wavelength of 410 nm and an emission wavelength of 480 nm.

Why study histone demethylases?

Histone methylation is central to many aspects of the biology of the nucleus, including transcriptional regulation of the control of higher order chromatin structure. Histone methyltransferases (HMTs) and histone demethylases (HDMs) work in opposition to each other to regulate histone methylation. Studying the activity of these enzymes gives insight into the mechanisms by which transcription and the organization of chromatin are regulated.

Assay advantages

- **Fast** – complete assay in 2.5 hours
- **Recombinant substrate** – more closely resembles *in vivo* conditions
- **Controls included** – LSD1 as a positive control and a formaldehyde standard
- **Complete assay** – optimized buffers for enhanced enzymatic activity

For more information about our fluorescent Histone Demethylase Assay, please visit us at www.activemotif.com/lsd1.

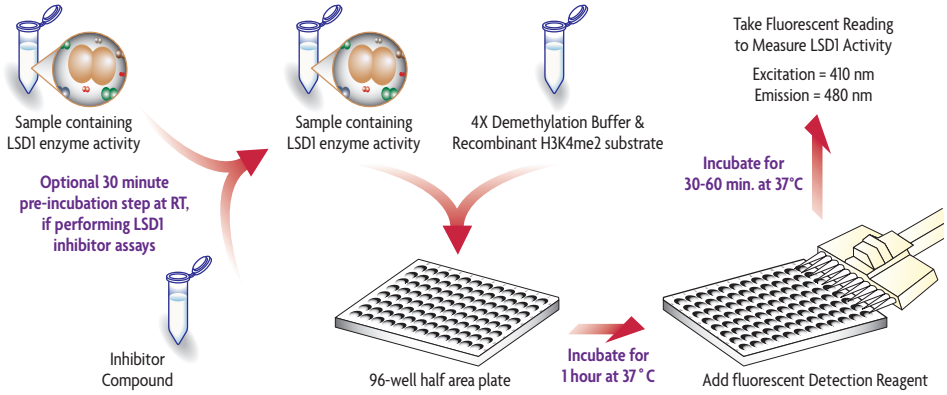

Figure 1: Schematic representation of the fluorescent Histone Demethylase Assay procedure.
If you are testing an LSD1 Inhibitor, it is pre-incubated with the enzyme sample. This, or the sample alone, is incubated for an hour with the H3K4Me2 substrate and buffer in a microplate, during which demethylation occurs. Detection Reagent is then added, and fluorescence is measured on a microplate reader, which quantitates the demethylase activity.

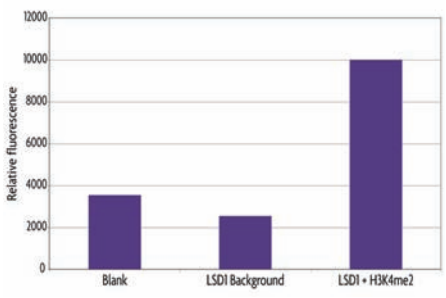

Figure 2: The Histone Demethylase Assay was used to assay activity of the LSD1 positive control.
LSD1 (1 µg) was tested in the absence or presence of the recombinant histone substrate. The enzymatic reaction was incubated at 37°C for one hour, followed by a one hour incubation with the Detection Reagent before the fluorescence intensity was measured.

Product	Format	Catalog No.
Histone Demethylase Assay (Fluorescent)	48 rxns	53200