

#4064 Store at -20°C

LSD1 (1B2E5) Mouse mAb

✓ 100 µl (10 Western mini-blot)



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New10/06

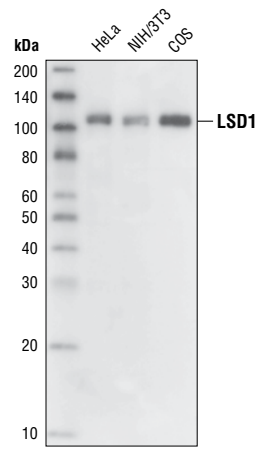
This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity	Molecular Wt.	Source	Isotype
W	H, M, R, Mk	110 kDa	Mouse	IgG1

Background: Lysine-specific demethylase 1 (LSD1; also known as AOF2 and BHC110) is a nuclear homolog of amine oxidases and functions as a histone demethylase and transcription cofactor (1). Gene activation and repression is specifically regulated by the methylation state of distinct lysine residues in histone proteins. For example, methylation of histone H3 Lys4 facilitates transcriptional activation by coordinating the recruitment of BPTF, a component of the NURF chromatin remodeling complex, and WDR5, a component of multiple histone methyltransferase complexes (2,3). In contrast, methylation of histone H3 Lys9 facilitates transcriptional repression by recruiting HP1 (4,5). LSD1 is a component of the CoREST transcriptional co-repressor complex, which contains CoREST, CtBP, HDAC1 and HDAC2. As part of this complex, LSD1 demethylates mono-methyl and di-methyl histone H3 Lys4 via an FAD-dependent oxidation reaction, and facilitates the repression of neuronal-specific genes in non-neuronal cells (1,6,7). In contrast, when LSD1 is associated with androgen-receptor in human prostate cells, it demethylates mono-methyl and di-methyl histone H3 Lys9 and facilitates androgen-receptor-dependent transcriptional activation (8). Therefore, LSD1 can function as a co-repressor or co-activator, depending on gene context. LSD1 activity is inhibited by the amine oxidase inhibitors pargyline, deprenyl, clorgyline and tranylcypromine (8).

Specificity/Sensitivity: LSD1 (1B2E5) Mouse mAb detects endogenous levels of total LSD1 protein.

Source/Purification: Monoclonal antibodies are produced by immunizing mice with recombinant human LSD1 protein.



Western blot analysis of cell lysates from HeLa, NIH/3T3 and COS cells, using LSD1 (1B2E5) Mouse mAb.

Background References:

- (1) Shi, Y. et al. (2004) *Cell* 119, 941–953.
- (2) Wysocka, J. et al. (2006) *Nature* 442, 86–90.
- (3) Wysocka, J. et al. (2005) *Cell* 121, 859–872.
- (4) Jacobs, S.A. and Khorasanizadeh, S. (2002) *Science* 295, 2080–2083.
- (5) Nielsen, P.R. et al. (2002) *Nature* 416, 103–107.
- (6) Shi, Y.J. et al. (2005) *Mol. Cell* 19, 857–864.
- (7) Lee, M.G. et al. (2005) *Nature* 437, 432–435.
- (8) Metzger, E. et al. (2005) *Nature* 437, 436–439.

Storage: Supplied in 10 mM HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

Recommended Antibody Dilutions:
Western blotting 1:1000

- Companion Products:**
- LSD1 Antibody #2139
 - Mono-Methyl Histone H3 (Lys4) Antibody #9723
 - Tri-Methyl Histone H3 (Lys4) Antibody #9727
 - Histone H3 Antibody #9715
 - Histone H3 (3H1) Rabbit mAb #9717
 - Histone Deacetylase 1 (HDAC1) Antibody #2062
 - Phototope®-HRP Western Blot Detection System, Anti-mouse IgG, HRP-linked Antibody #7072
 - Anti-mouse IgG, HRP-linked Antibody #7076
 - Prestained Protein Marker, Broad Range (Premixed Format) #7720
 - Biotinylated Protein Ladder Detection Pack #7727
 - 20X LumiGLO® Reagent and 20X Peroxide #7003

IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish B—bovine All—all species expected
 F—Flow cytometry E—ELISA D—DELFIATM
 Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Western Immunoblotting Protocol (Primary Ab Incubation In Milk)

For Western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% nonfat dry milk; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g nonfat dry milk and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope®-HRP Western Blot Detection System #7072:** Includes biotinylated protein ladder, secondary anti-mouse (#7076) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight marker (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.