#8694 Store at -20°C

Lysine Methyltransferase Antibody Sampler Kit

☑ 1 Kit
(8 x 20 µl)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
ASH2L (D93F6) XP® Rabbit mAb	5019	20 µl	80, 65 kDa	Rabbit IgG
ESET (C1C12) Rabbit mAb	2196	20 µl	180 kDa	Rabbit IgG
G9a/EHMT2 (C6H3) Rabbit mAb	3306	20 µl	160, 180 kDa	Rabbit IgG
RBBP5 (D3I6P) Rabbit mAb	13171	20 µl	70 kDa	Rabbit IgG
SET7/SET9 Antibody	2813	20 µl	48 kDa	Rabbit IgG
SET8 (C18B7) Rabbit mAb	2996	20 µl	43 kDa	Rabbit IgG
SMYD2 (D14H7) Rabbit mAb	9734	20 µl	49 kDa	Rabbit IgG
SUV39H1 (D11B6) Rabbit mAb	8729	20 µl	48 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Storage: Supplied in 10 mM sodium 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 antibodies.*

Recommended Antibody Dilutions:
Western blotting 1:1000

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

Description: The Lysine Methyltransferase Antibody Sampler Kit provides a fast and economical means to evaluate endogenous levels of lysine methyltransferases. The kit includes enough antibody to perform two western blot experiments with each per primary antibody.

Background: SET domain-containing proteins are potential histone methyltransferases (HMTases), which are classified into subgroups by their putative substrate specificities. Histone H3 Lys9 (H3-K9) methyltransferase group genes include Suv39h1, Suv39h2, G9a, G9a related protein (GLP) and SETDB1/ESET (1). The H3-K9 methylation mark plays an important role as a binding site for the chromo-containing protein, resulting in chromatin compaction and heterochromatin generation (2). Histone H3-K4 methylation is exclusively associated with actively transcribed genes (2). The first H3-K4 methylase complex, COMPASS, was identified in the yeast S. cerevisiae and consists of Set1/KMT2 and seven other polypeptides, Cps60-Cps15 (2). Set1/KMT2 functions within COMPASS and is capable of mono-, di-, and trimethylating H3-K4 (2). There are several Set1 related proteins in mammals including WDR5, RBBP5, ASH2L, CXXC1, and DPY30 (2,3). SET7/SET9 is a member of the SET domain-containing family that can specifically methylate H3-K4, Lys189 of the TAF10, a member of the TFIID transcription factor complex, and Lys372 of the p53 tumor suppressor protein (4-6). SET domain-containing lysine methyltransferase 8 (SET8), also known as PR/SET domain-containing protein 7 (PR/SET7), is a single-subunit enzyme that mono-methylates histone H4-K20, preferably on nucleosomal substrates (7-9). SET and MYND domain-containing protein 2 (SMYD2), also known as lysine methyltransferase protein 3C (KMT3C), functions to repress transcription by interacting with the Sin3A repressor complex and methylating H3-K36 (10). SMYD2 also methylates H3-K4 through interaction with HSP90α, and methylates p53 at Lys370 to repress p53-mediated transcriptional activation and apoptosis (11,12).

Specificity/Sensitivity: Each antibody in this kit recognizes only the specific target protein and does not cross-react with other family members.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to the sequence of human ASH2L protein, the carboxy terminus of human ESET protein, the carboxy terminus of human G9a/EHMT2 protein, human SET8 protein, residues surrounding Val414 of human SMYD2 protein, residues surrounding Val408 of human RBBP5 protein, or residues surrounding Asp380 of human SUV39H1 protein. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues the human SET7/SET9 protein. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Background References:

(1) Tachibana, M. et al. (2005) *Genes Dev* 19, 815-26.
(2) Shilatifard, A. (2008) *Curr Opin Cell Biol* 20, 341-8.
(3) Lee, J.H. et al. (2007) *J Biol Chem* 282, 13419-28.
(4) Nishioka, K. et al. (2002) *Genes Dev* 16, 479-89.
(5) Kouskouti, A. et al. (2004) *Mol Cell* 14, 175-82.
(6) Chuikov, S. et al. (2004) *Nature* 432, 353-60.
(7) Fang, J. et al. (2002) *Curr Biol* 12, 1086-99.
(8) Xiao, B. et al. (2005) *Genes Dev* 19, 1444-54.
(9) Couture, J.F. et al. (2005) *Genes Dev* 19, 1455-65.
(10) Brown, M.A. et al. (2006) *Mol Cancer* 5, 26.
(11) Abu-Farha, M. et al. (2008) *Mol Cell Proteomics* 7, 560-72.
(12) Huang, J. et al. (2006) *Nature* 444, 629-32.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST

Bovine Serum Albumin (BSA): (#9998)

Primary Antibody Dilution Buffer: 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.

Biotinylated Protein Ladder Detection Pack: (#7727)

Prestained Protein Marker, Broad Range (Premixed Format): (#7720)

Blotting Membrane and Paper: (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.

Secondary Antibody Conjugated to HRP: anti-rabbit (#7074); anti-mouse (#7076)

Detection Reagent: LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.