Store at -20°C

Methyl-Histone H3 (Lys79) Antibody Sampler Kit

#45139

1 Kit
(4 x 20 µl)

New 10/18

Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/support

Orders: 877-616-2355 (U.S.)
orders@cellsignal.com

Entrez-Gene ID #8350
UniProt ID #P68431

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype/Source
Tri-Methyl-Histone H3 (Lys79) (E8B3M) Rabbit mAb	74073	20 µl	17 kDa	Rabbit IgG
Di-Methyl-Histone H3 (Lys79) (D15E8) XP® Rabbit mAb	5427	20 µl	17 kDa	Rabbit IgG
Mono-Methyl-Histone H3 (Lys79) (D5X1S) Rabbit mAb	12522	20 µl	17 kDa	Rabbit IgG
Histone H3 (D1H2) XP® Rabbit mAb	4499	20 µl	17 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.

Description: The Methyl-Histone H3 (Lys79) Antibody Sampler Kit provides an economical means of detecting levels of mono-, di-, and tri-methyl histone H3 Lys79 using methyl-specific and control histone H3 antibodies. The kit contains enough primary antibodies to perform at least two western blot experiments.

Background: The nucleosome, made up of four core histone proteins (H2A, H2B, H3, and H4), is the primary building block of chromatin. Originally thought to function as a static scaffold for DNA packaging, histones have now been shown to be dynamic proteins, undergoing multiple types of post-translational modifications, including acetylation, phosphorylation, methylation, and ubiquitination (1). Histone methylation is a major determinant for the formation of active and inactive regions of the genome and is crucial for the proper programming of the genome during development (2,3). Arginine methylation of histones H3 (Arg2, 17, 26) and H4 (Arg3) promotes transcriptional activation and is mediated by a family of protein arginine methyltransferases (PRMTs), including the co-activators PRMT1 and CARM1 (PRMT4) (4). In contrast, a more diverse set of histone lysine methyltransferases has been identified, all but one of which contain a conserved catalytic SET domain originally identified in the *Drosophila* Su(var)3-9, Enhancer of zeste, and Trithorax proteins. Lysine methylation occurs primarily on histones H3 (Lys4, 9, 27, 36, 79) and H4 (Lys20) and has been implicated in both transcriptional activation and silencing (4). Methylation of these lysine residues coordinates the recruitment of chromatin modifying enzymes containing methyl-lysine binding modules such as chromodomains (HP1, PRC1), PHD fingers (BPTF, ING2), tudor domains (53BP1), and WD-40 domains (WDR5) (5-8). The discovery of histone demethylases such as PADI4, LSD1, JMJD1, JMJD2, and JHDM1 has shown that methylation is a reversible epigenetic marker (9). Methylation of histone H3 Lys79 is mediated by the DOT1L histone methyltransferase, is associated with transcriptionally active genes, and plays a role in the regulation of DNA damage response, cell cycle, and embryonic stem cell development.

Specificity/Sensitivity: Each antibody in the Methyl-Histone H3 (Lys79) Antibody Sampler Kit detects endogenous levels of its target protein. Tri-Methyl-Histone H3 (Lys79) (E8B3M) Rabbit mAb recognizes endogenous levels of histone H3 protein when tri-methylated at Lys79. This antibody shows some cross-reactivity to histone H3 that is di-methylated on Lys79, but does not cross-react with non-methylated or mono-methylated histone H3 Lys79. Di-Methyl-Histone H3 (Lys79) (D15E8) XP® Rabbit mAb detects endogenous levels of histone H3 only when di-methylated on Lys79. Mono-Methyl-Histone H3 (Lys79) (D5X1S) Rabbit mAb recognizes endogenous levels of histone H3 protein only when mono-methylated at Lys79. These methyl-Lys79 antibodies do not cross-react with histone H3 methylated at Lys4, Lys9, Lys27, or Lys36. Histone H3 (D1H2) XP® Rabbit mAb detects endogenous levels of total Histone H3 protein, including isoforms H3.1, H3.2, H3.3, and the variant histone CENP-A. This antibody does not cross-react with other core histones.

Source/Purification: Monoclonal methyl-histone H3 Lys79 antibodies are produced by immunizing rabbits with synthetic peptides corresponding to the amino terminus of histone H3 in which Lys79 is mono-, di-, or tri-methylated. The control histone H3 monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to the carboxy terminus of the human histone H3 protein.

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 antibody.*

Recommended Antibody Dilutions:

Western blotting	1:1000

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com.

Background References:

(1) Peterson, C.L. and Laniel, M.A. (2004) *Curr Biol* 14, R546-51.
(2) Kubicek, S. et al. (2006) *Ernst Schering Res Found Workshop* , 1-27.
(3) Lin, W. and Dent, S.Y. (2006) *Curr Opin Genet Dev* 16, 137-42.
(4) Lee, D.Y. et al. (2005) *Endocr Rev* 26, 147-70.
(5) Daniel, J.A. et al. (2005) *Cell Cycle* 4, 919-26.
(6) Shi, X. et al. (2006) *Nature* 442, 96-9.
(7) Wysocka, J. et al. (2006) *Nature* 442, 86-90.
(8) Wysocka, J. et al. (2005) *Cell* 121, 859-72.
(9) Trojer, P. and Reinberg, D. (2006) *Cell* 125, 213-7.

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **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.

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
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **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.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **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.