Store at -20°C

#9788

Polycomb Group Antibody Sampler Kit

☑ 1 Kit
(6 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
SUZ12 (D39F6) XP® Rabbit mAb	3737	20 µl	83 kDa	Rabbit IgG
Ezh2 (D2C9) XP® Rabbit mAb	5246	20 µl	98 kDa	Rabbit IgG
Ring1A (D2P4D) Rabbit mAb	13069	20 µl	54 kDa	Rabbit IgG
RING1B (D22F2) XP® Rabbit mAb	5694	20 µl	41 kDa	Rabbit IgG
Bmi1 (D20B7) XP® Rabbit mAb	6964	20 µl	41, 43 kDa	Rabbit IgG
Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb	9733	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.

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 Polycomb Group Proteins Antibody Sampler Kit provides an economical means of evaluating total levels of Polycomb Group Proteins. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: The polycomb group (PcG) proteins contribute to the maintenance of cell identity, stem cell self-renewal, cell-cycle regulation, and oncogenesis by maintaining the silenced state of genes that promote cell lineage specification, cell death, and cell-cycle arrest (1-4). PcG proteins exist in two complexes that cooperate to maintain long-term gene silencing through epigenetic chromatin modifications. The first complex, Eed-Ezh2, is recruited to genes by DNA-binding transcription factors and methylates histone H3 on Lys27. This histone methyltransferase activity requires the Ezh2, Eed, and Suz12 subunits of the complex (5). Methylation of Lys27 facilitates the recruitment of the second complex, PRC1, which ubiquitinates histone H2A on Lys119 (6). PRC1 is composed of Bmi1 and RING1A (also RING1 or RNF1), both of which act to enhance the E3 ubiquitin ligase activity of an additional catalytic subunit RING1B (also RING2 or RNF2) (7). PcG proteins play an important role in the regulation of cell proliferation and senescence through repression of the p16 INK4A and p19 ARF genes and are required for maintenance of adult hematopoietic and neural stem cells, as well as embryonic stem cells (3,4,8-10).

Specificity/Sensitivity: Each antibody in the Polycomb Group Proteins Antibody Sampler Kit detects endogenous levels of its target protein.

Source/Purification: Monoclonal antibody is produced by immunizing animals with synthetic peptides corresponding to residues surrounding Arg354 of human Ezh2, Pro316 of human Ring1A protein, the human SUZ12 protein, the carboxy terminus of human Bmi1 protein, the amino terminus of histone H3 in which Lys27 is tri-methylated, or with recombinant protein specific to the full-length human RING1B protein, respectively.

Background References:

(1) Boyer, L.A. et al. (2006) *Nature* 441, 349-53.
(2) Lee, T.I. et al. (2006) *Cell* 125, 301-13.
(3) Park, I.K. et al. (2003) *Nature* 423, 302-5.
(4) Molofsky, A.V. et al. (2003) *Nature* 425, 962-7.
(5) Cao, R. and Zhang, Y. (2004) *Mol Cell* 15, 57-67.
(6) Wang, H. et al. (2004) *Nature* 431, 873-8.
(7) Cao, R. et al. (2005) *Mol Cell* 20, 845-54.
(8) Molofsky, A.V. et al. (2005) *Genes Dev* 19, 1432-7.
(9) Jacobs, J.J. et al. (1999) *Nature* 397, 164-8.
(10) Jacobs, J.J. et al. (1999) *Genes Dev* 13, 2678-90.

U.S. Patent No. 5,675,063

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 **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
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.