Store at -20°C

#62083

# Polycomb Group 2 (PRC2) Antibody Sampler Kit

1 Kit
(6 x 20 µl)

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

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

rev. 04/10/19

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

| Products Included | Product # | Quantity | Mol. Wt. | Isotype/Source |
|---|---|---|---|---|
| Ezh2 (D2C9) XP® Rabbit mAb | 5246 | 20 µl | 98 kDa | Rabbit IgG |
| EZH1 (D7D5D) Rabbit mAb | 42088 | 20 µl | 95 kDa | Rabbit IgG |
| SUZ12 (D39F6) XP® Rabbit mAb | 3737 | 20 µl | 83 kDa | Rabbit IgG |
| EED Rabbit Antibody | 51673 | 20 µl | 50-70 kDa | Rabbit |
| JARID2 (D6M9X) Rabbit mAb | 13594 | 20 µl | 150 kDa | Rabbit IgG |
| AEBP2 (D7C6X) Rabbit mAb | 14129 | 20 µl | 28, 70 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 Polycomb Group 2 (PRC2) Antibody Sampler Kit provides an economical means of evaluating total levels of Polycomb Group 2 Proteins. The kit contains enough primary and secondary antibodies to perform two western blot experiments.

**Background:** The polycomb group (PcG) proteins are involved in maintaining the silenced state of multiple developmentally regulated genes and contribute to the maintenance of cell identity, cell cycle regulation, and oncogenesis (1-4). Enhancer of zest homolog 1 (Ezh1) and enhancer of zest homolog 2 (Ezh2) are members of this large protein family and are subunits of the polycomb repressor complex 2 (PRC2), also known to contain SUZ12, EED, JARID2, and AEBP2. Ezh1 and its paralog Ezh2 are mutually exclusive catalytic subunits of the PRC2 complex, which functions to mono-, di-, and tri-methylate Lys27 on histone H3, all marks that are associated with transcriptional repression. SUZ12 and EED proteins are also absolutely required for methyltransferase activity (5). JARID2 and AEBP2 are both accessory proteins that function to recruit the PRC2 complex to target genes and enhance methyltransferase activity by binding to DNA and histone proteins in nucleosomes (6-14).

**Specificity/Sensitivity:** Each antibody in the Polycomb Group 2 (PRC2) Antibody Sampler Kit detects endogenous levels of target proteins.

**Source/Purification:** Monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues surrounding Arg354 of human Ezh2, Pro257 of human SUZ12 protein, Leu23 of human EED, Asp1114 of human JARID2, Leu345 of human AEBP2, and the carboxy terminus of EZH1.

**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 |

**Background References:**

(1) Boyer, L.A. et al. (2006) *Nature* 441, 349-53.
(2) Cao, R. et al. (2002) *Science* 298, 1039-43.
(3) Müller, J. et al. (2002) *Cell* 111, 197-208.
(4) Lee, T.I. et al. (2006) *Cell* 125, 301-13.
(5) Cao, R. and Zhang, Y. (2004) *Mol Cell* 15, 57-67.
(6) Peng, J.C. et al. (2009) *Cell* 139, 1290-302.
(7) Shen, X. et al. (2009) *Cell* 139, 1303-14.
(8) Pasini, D. et al. (2010) *Nature* 464, 306-10.
(9) Li, G. et al. (2010) *Genes Dev* 24, 368-80.
(10) Son, J. et al. (2013) *Genes Dev* 27, 2663-77.
(11) Kaneko, S. et al. (2014) *Mol Cell* 53, 290-300.
(12) Cao, R. and Zhang, Y. (2004) *Mol Cell* 15, 57-67.
(13) Kim, H. et al. (2009) *Nucleic Acids Res* 37, 2940-50.
(14) Kalb, R. et al. (2014) *Nat Struct Mol Biol* 21, 569-71.

Tween is a registered trademark of ICI Americas, Inc.

Thank you for your recent purchase. If you would like to provide a review visit cellsignal.com/comments.

www.cellsignal.com

© 2018 Cell Signaling Technology, Inc.
XP and Cell Signaling Technology are trademarks of Cell Signaling Technology, Inc.



**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.

LumiGLO is a registered trademark of Kirkegaard & Perry Laboratories. Tween is a registered trademark of ICI Americas, INC. SignalFire is a trademark of Cell Signaling Technology, INC.



**Orders:** 877-616-2355 | orders@cellsignal.com | **Support:** +1-978-867-2388 | www.cellsignal.com/support

© 2017 Cell Signaling Technology, Inc. Cell Signaling Technology® is a trademark of Cell Signaling Technology, Inc.