#5694 Store at -20°C

RING1B (D22F2) XP® Rabbit mAb

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

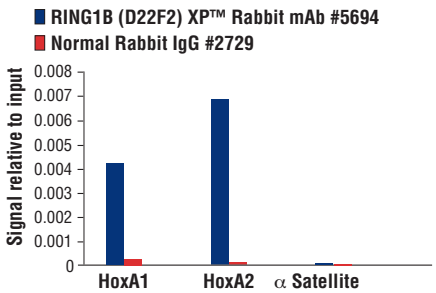
rev. 01/26/17

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

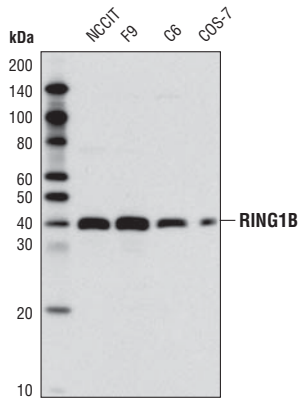
Entrez-Gene ID #6045
UniProt ID #Q99496

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IF-IC, ChIP, ChIP-seq, F Endogenous	H, M, R, Mk	41 kDa	Rabbit IgG**

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).



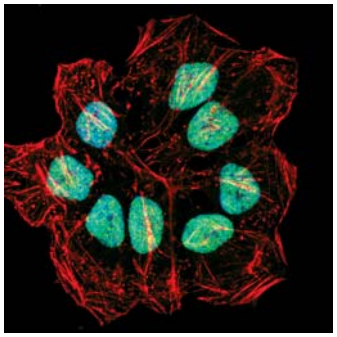
Chromatin immunoprecipitations were performed with cross-linked chromatin from 4 x 10^6 NCCIT cells and either 10 µl of RING1B (D22F2) XP® Rabbit mAb or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human HoxA1 Intron 1 Primers #7707, SimpleChIP® Human HoxA2 Promoter Primers #5517, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin (equivalent to one).



Western blot analysis of extracts from various cell lines using RING1B (D22F2) XP® Rabbit mAb.

Specificity/Sensitivity: RING1B (D22F2) XP® Rabbit mAb recognizes endogenous levels of total RING1B protein.

Source/Purification: Monoclonal antibody is produced by immunizing animals with recombinant protein specific to the full-length human RING1B protein.



Confocal immunofluorescent analysis of HeLa cells using RING1B (D22F2) XP® Rabbit mAb (green). Actin filaments were labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

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

***Species cross-reactivity is determined by western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunofluorescence (IF-IC)	1:500
Chromatin IP	1:50
Chromatin IP-seq	1:50
Immunoprecipitation	1:200
Flow Cytometry	1:400

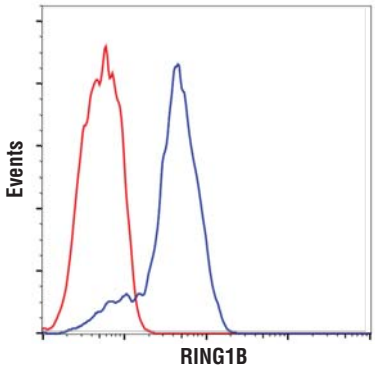
For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

Background References:

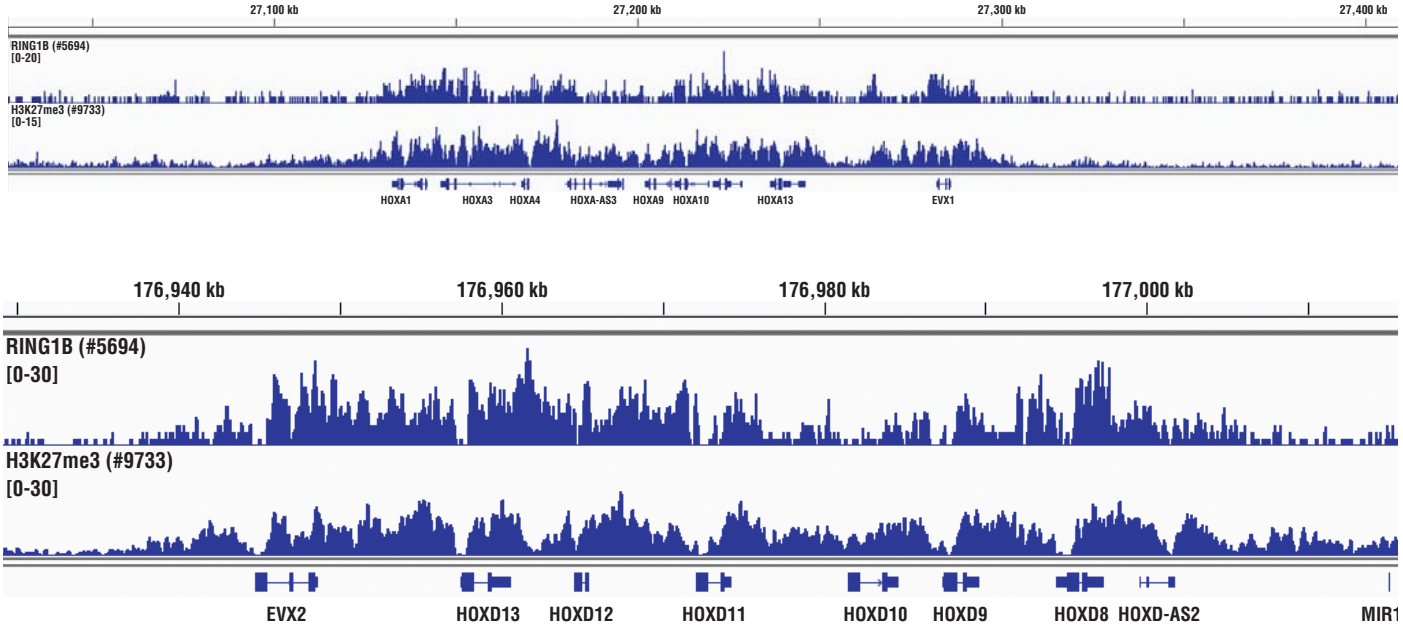
(1) Boyer, L.A. et al. (2006) *Nature* 441, 349-53.
(2) Lee, T.I. et al. (2006) *Cell* 125, 301-13.
(3) Park, I.K. et al. (2003) *Nature* 423, 302-5.
(4) Molofsky, A.V. et al. (2003) *Nature* 425, 962-7.
(5) Cao, R. and Zhang, Y. (2004) *Mol Cell* 15, 57-67.
(6) Wang, H. et al. (2004) *Nature* 431, 873-8.
(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, 2678-9

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

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.



Flow cytometric analysis of HeLa cells using RING1B (D22F2) XP® Rabbit mAb (blue) compared to concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (red). Anti-rabbit IgG (H+L), F(ab')$_2$ Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.



Chromatin immunoprecipitations were performed with cross-linked chromatin from 4 x 10^6 NCCIT cells and either 10 µl of RING1B (D22F2) XP® Rabbit mAb or 10 µl of Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb #9733, using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. DNA Libraries were prepared from 5ng enriched ChIP DNA for RING1B ChIP-seq and 50ng enriched ChIP DNA for H3K27me3 ChIP-seq using NEBNext® Ultra™ II DNA Library Prep Kit for Illumina®, and sequenced on the Illumina NextSeq. RING1B and H3K27me3 are known to associate with each other on chromatin. The figure shows binding of both RING1B and H3K27me3 across HOXA genes (upper) and HOXD genes (lower), which are known target genes of RING1B (see additional figure containing ChIP-qPCR data).