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Ezh2 (D2C9) XP® Rabbit mAb

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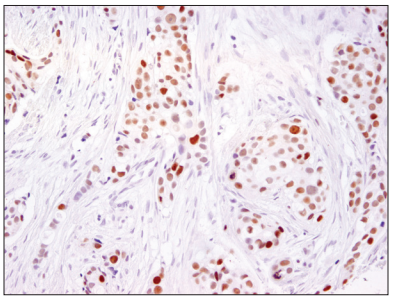
Entrez-Gene ID #2146
UniProt ID #Q15910

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P, IF-IC, IF-F, ChIP, ChIP-seq, F Endogenous	H, M, R, Mk	98 kDa	Rabbit IgG**

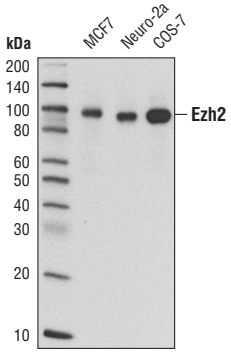
Background: The polycomb group (PcG) proteins are involved in maintaining the silenced state of several developmentally regulated genes and contribute to the maintenance of cell identity, cell cycle regulation, and oncogenesis (1,2). Enhancer of zeste homolog 2 (Ezh2), a member of this large protein family, contains four conserved regions including domain I, domain II, and a cysteine-rich amino acid stretch that precedes the carboxy-terminal SET domain (3). The SET domain has been linked with histone methyltransferase (HMTase) activity. Moreover, mammalian Ezh2 is a member of a histone deacetylase complex that functions in gene silencing, acting at the level of chromatin structure (4). Ezh2 complexes methylate histone H3 at Lys9 and 27 *in vitro*, which is thought to be involved in targeting transcriptional regulators to specific loci (5). Ezh2 is deregulated in various tumor types, and its role, both as a primary effector and as a mediator of tumorigenesis, has become a subject of increased interest (6).

Specificity/Sensitivity: Ezh2 (D2C9) XP® Rabbit mAb detects endogenous levels of total Ezh2 protein.

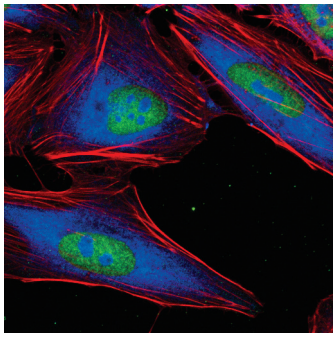
Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Arg354 of human Ezh2 protein.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma using Ezh2 (D2C9) XP® Rabbit mAb.



Western blot analysis of extracts from MCF7, Neuro-2a, and COS-7 cell lines using Ezh2 (D2C9) XP® Rabbit mAb.



Confocal immunofluorescent analysis of HeLa cells using Ezh2 (D2C9) XP® Rabbit mAb (green) and S6 Ribosomal Protein (54D2) Mouse mAb #2317 (blue). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red).

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.*

***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
Immunoprecipitation	1:300
Immunohistochemistry (Paraffin)	1:50†
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114

†*Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.*

Immunofluorescence (IF-IC)	1:200
Immunofluorescence (IF-F)	1:100
Chromatin IP	1:100
Chromatin IP-seq	1:100
Flow Cytometry	1:200

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) Seller, W.B. and Loda, M. (2002) *Cancer Cell* 2, 349-350.

(2) Visser, H.P. et al. (2001) *Br. J. Haematol.* 112, 950-958.

(3) Chen, H. et al. (1996) *Genomics* 38, 30-37.

(4) Tonini, T. et al. (2004) *Oncogene* 23, 4930-4937.

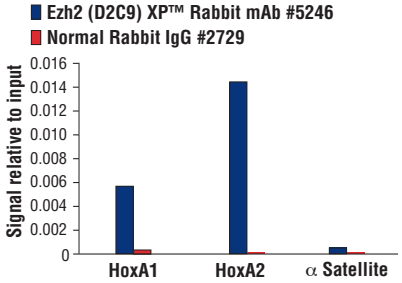
(5) Muller, J. et al. (2002) *Cell* 111, 197-208.

(6) Kleer, C.G. et al. (2003) *Proc Natl. Acad. Sci. USA* 100, 11606-11611.

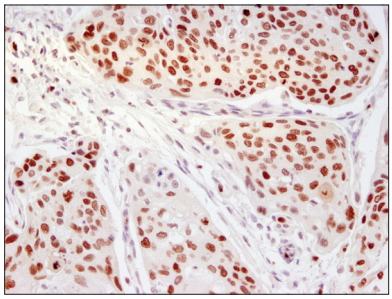
IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

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

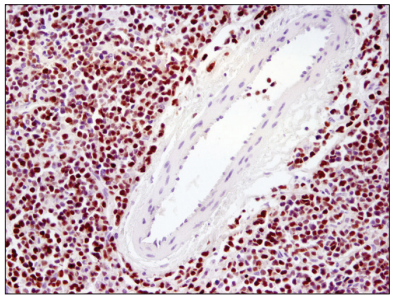
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



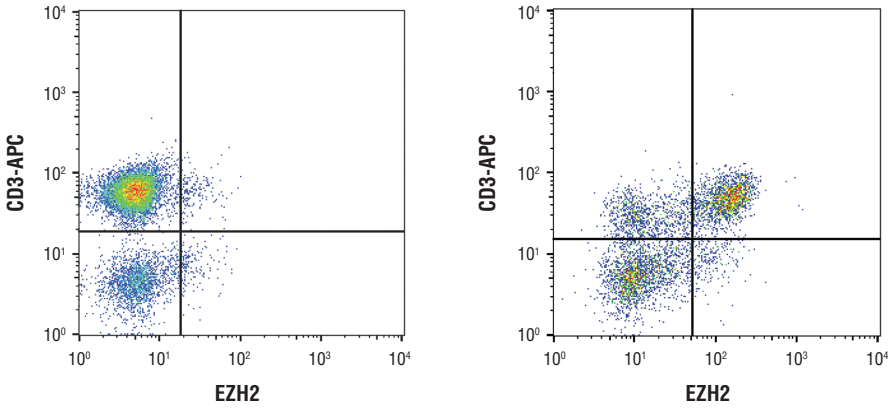
Chromatin immunoprecipitations were performed with cross-linked chromatin from 4 x 10^6 NCCIT cells and either 5 µl of Ezh2 (D2C9) XP® Rabbit mAb or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using 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, which is equivalent to one.



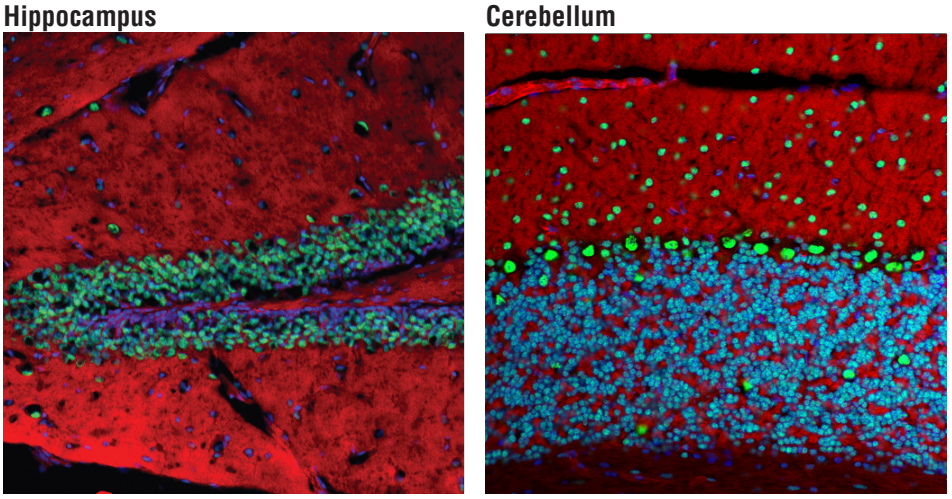
Immunohistochemical analysis of paraffin-embedded human cervical carcinoma using Ezh2 (D2C9) XP® Rabbit mAb.



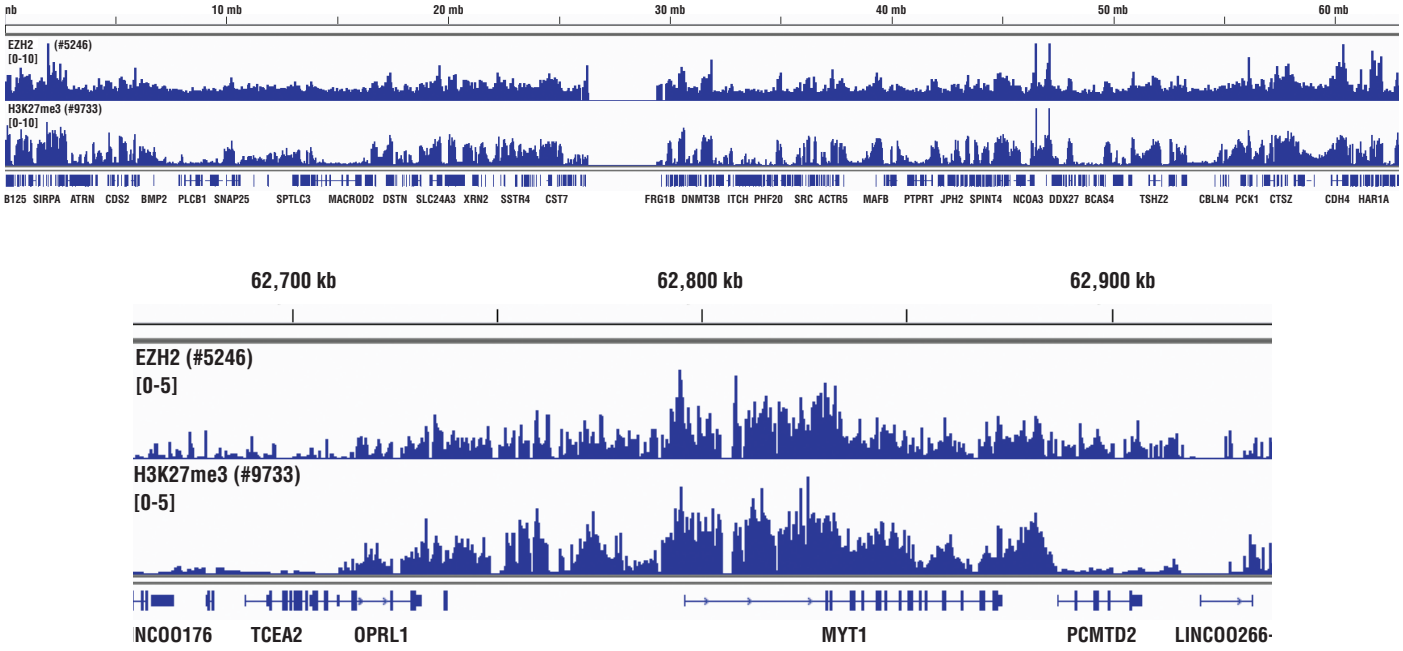
Immunohistochemical analysis of paraffin-embedded human lymphoma using Ezh2 (D2C9) XP® Rabbit mAb.



Flow cytometric analysis of human peripheral blood mononuclear cells untreated (left) and treated (right) with anti-human CD3 (10 µg/ml, coated plates) and anti-human CD28 (5 µg/ml) for 3 days at 37ºC using EZH2 (D2C9) XP® Rabbit mAb and co-stained with an anti-human CD3 antibody. Anti-rabbit IgG (H+L), F(ab')$_2$ Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.



Confocal immunofluorescent analysis of mouse hippocampus (left) and cerebellum (right) using Ezh2 (D2C9) XP® Rabbit mAb (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



Chromatin immunoprecipitations were performed with cross-linked chromatin from 4 x 10^6 Hela cells and either 5 µl of Ezh2 (D2C9) XP® Rabbit mAb or 10 µl of Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb, using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. DNA Libraries were prepared from 5ng enriched ChIP DNA for EZH2 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. EZH2 and H3K27me3 are known to associate with each other on chromatin. The figure shows binding of both EZH2 and H3K27me3 across chromosome 20 (upper), including MYT1 gene (lower).