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#14893

MLLT1/ENL (D9M4B) Rabbit mAb

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Entrez-Gene ID #4298
UniProt ID #Q03111

rev. 07/31/18

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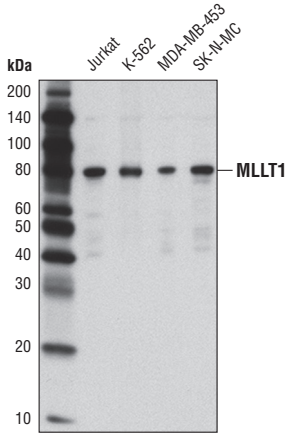
Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, ChIP, ChIP-seq	H	80 kDa	Rabbit IgG**
Endogenous			

Background: The super elongation complex (SEC) plays a critical role in regulating RNA polymerase II (RNAPII) transcription elongation (1). The SEC is composed of AFF4, AFF1/AF4, MLLT3/AF9, and MLLT1/ENL proteins. The pathogenesis of mixed lineage leukemia is often associated with translocations of the SEC subunits joined to the histone H3 Lys4 methyltransferase mixed lineage leukemia (MLL) gene (1-4). The SEC has been found to contain RNAPII elongation factors eleven-nineteen lysine-rich leukemia (ELL), ELL2, and ELL3, along with the associated factors EAF1 and EAF2, which can increase the catalytic rate of RNAPII transcription *in vitro*, (1,2,5-7). The SEC positive transcription elongation factor b (P-TEFb) phosphorylates the carboxy-terminal domain within the largest subunit of RNAP II at Ser2 of the heptapeptide repeat. The SEC negative transcription elongation factors, DRB-induced stimulating factor (DSIF) and negative elongation factor (NELF), signal the transition from transcription initiation and pausing to productive transcription elongation (2,8-10). The chromosomal translocation of MLL with the members of the SEC leads to SEC recruitment to MLL regulated genes, such as the highly developmentally regulated Hox genes, implicating the misregulation and overexpression of these genes as underlying contributors to leukemogenesis (1,2,9,11).

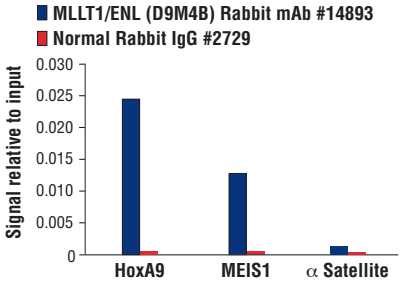
MLL translocated to 1/eleven-nineteen-leukemia (MLLT1/ENL) is also found as part of the histone H3 Lys79 methyltransferase disruptor of telomeric silencing-like (Dot1L) complex that has been suggested to play a role in transcription elongation. This complex regulates the expression of genes, such as the Wnt-signaling pathway target genes that control cell proliferation and differentiation during development (12,13).

Specificity/Sensitivity: MLLT1/ENL (D9M4B) Rabbit mAb recognizes endogenous levels of total MLLT1/ENL protein.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ala343 of human MLLT1/ENL protein.



Western blot analysis of extracts from various cell lines using MLLT1/ENL (D9M4B) Rabbit mAb.



Chromatin immunoprecipitations were performed with cross-linked chromatin from MV-4-11 cells and either MLLT1/ENL (D9M4B) Rabbit mAb or Normal Rabbit IgG #2729, using SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human HoxA9 Promoter Primers #14909, human MEIS1 promoter primers, 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.

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
Chromatin IP / Chromatin IP-seq	1:50

Optimal ChIP / ChIP-seq conditions: 10 µl of antibody & 10 µg of chromatin (4 x 10^6 cells) per IP. Antibody validated using SimpleChIP® Enzymatic ChIP Kits.

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) Mohan, M. et al. (2010) *Nat Rev Cancer* 10, 721-8.
(2) Lin, C. et al. (2010) *Mol Cell* 37, 429-37.
(3) Drexler, H.G. et al. (2004) *Leukemia* 18, 227-32.
(4) Smith, E. et al. (2011) *Genes Dev* 25, 661-72.
(5) Shilatifard, A. et al. (1996) *Science* 271, 1873-6.
(6) Shilatifard, A. et al. (1997) *Proc Natl Acad Sci USA* 94, 3639-43.
(7) Miller, T. et al. (2000) *J Biol Chem* 275, 32052-6.
(8) Lin, C. et al. (2011) *Genes Dev* 25, 1486-98.
(9) Yokoyama, A. et al. (2010) *Cancer Cell* 17, 198-212.
(10) Cho, S. et al. (2010) *Cell Cycle* 9, 1697-705.
(11) Shah, N. and Sukumar, S. (2010) *Nat Rev Cancer* 10, 361-71.
(12) Mohan, M. et al. (2010) *Genes Dev* 24, 574-89.
(13) Nguyen, A.T. and Zhang, Y. (2011) *Genes Dev* 25, 1345-58.

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.

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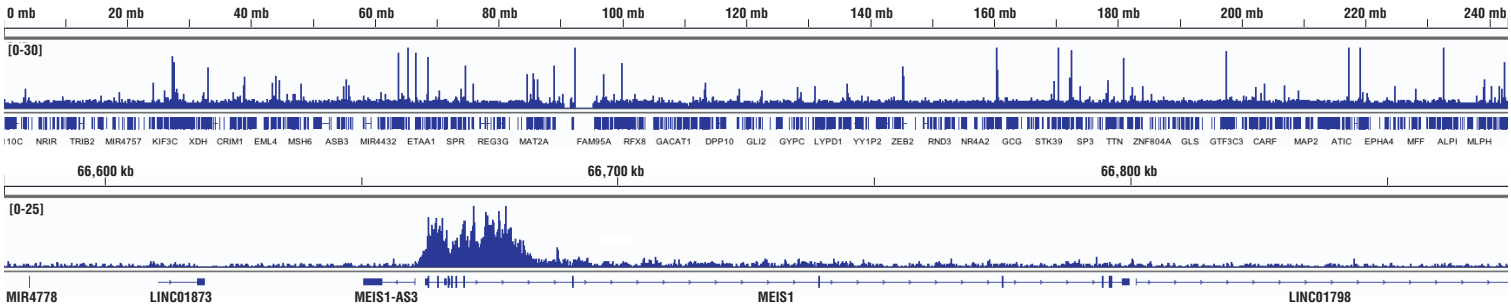
Cell Signaling TECHNOLOGY®

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.



Chromatin immunoprecipitations were performed with cross-linked chromatin from MV-4-11 cells and MLLT1/ENL (D9M4B) Rabbit mAb, using SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. DNA Libraries were prepared using SimpleChIP® ChIP-seq DNA Library Prep Kit for Illumina® #56795. The figure shows binding across chromosome 2 (upper), including MEIS1 (lower), a known target gene of MLLT1/ENL (see additional figure containing ChIP-qPCR data).