

Store at
-20°C

#14129

AEBP2 (D7C6X) Rabbit mAb

www.cellsignal.com

Support: 877-678-TECH (8324)
www.cellsignal.com/supportOrders: 877-616-CELL (2355)
orders@cellsignal.comEntrez-Gene ID #121536
UniProt ID #Q6ZLN18

rev. 10/29/18

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

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, ChIP, ChIP-seq Endogenous	H, M, R, Mk, (Hm, C, B)	70, 28 kDa	Rabbit IgG**

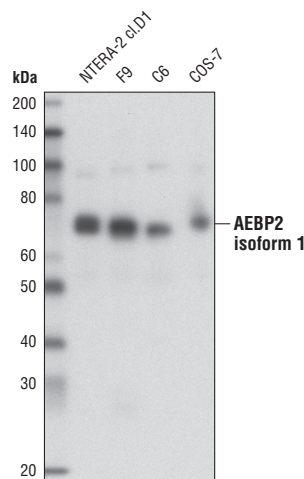
Background: Polycomb group (PcG) proteins contribute to the maintenance of cell identity, stem cell self-renewal, cell cycle regulation, and oncogenesis by maintaining the silenced state of genes that promote cell lineage specification, cell death, and cell-cycle arrest (1-4). PcG proteins exist in two complexes that cooperate to maintain long-term gene silencing through epigenetic chromatin modifications. The PRC2 (EZH2-EED) complex is recruited to genes by DNA-binding transcription factors and methylates histone H3 on Lys27. Methylation of Lys27 facilitates the recruitment of the PRC1 complex, which ubiquitinylates histone H2A on Lys119 (5). Suppressor of Zeste 12 (SUZ12) is an obligate component of the PRC2 complex, which together with EZH2 and EED is absolutely required for histone methyltransferase activity of the protein complex (6).

The zinc finger AE binding protein 2 (AEBP2) is another integral component of the PRC2 complex. Addition of AEBP2 to the PRC2 core complex (EZH2-EED-SUZ12) enhances histone H3 Lys27 methyltransferase activity on nucleosomal substrates *in vitro*, which may be mediated in part by three AEBP2 DNA-binding zinc finger domains (5,7). AEBP2-mediated enhancement of enzymatic activity is greater on nucleosomal substrates that contain mono-ubiquitinated histone H2A Lys119, which suggests that AEBP2 may target PRC2 complexes *in vivo* through binding to DNA and mono-ubiquitinated histone H2A Lys119 (8).

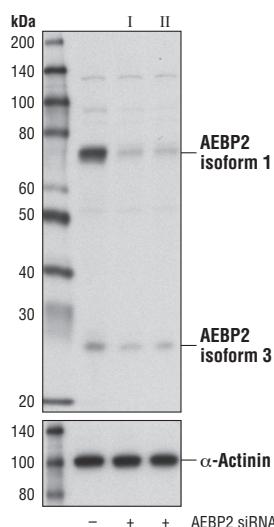
Specificity/Sensitivity: AEBP2 (D7C6X) Rabbit mAb recognizes endogenous levels of total AEBP2 protein.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Leu345 of human AEBP2 protein.

Western blot analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® AEBP2 siRNA I #14711 (+), or SignalSilence® AEBP2 siRNA II #14713 (+), using AEBP2 (D7C6X) Rabbit mAb (upper) or α -Actinin (D6F6) XP® Rabbit mAb #6487 (lower). The AEBP2 (D7C6X) Rabbit mAb confirms silencing of AEBP2 expression, while the α -Actinin (D6F6) XP® Rabbit mAb is used as a loading control.



Western blot analysis of extracts from various cell lines using AEBP2 (D7C6X) Rabbit mAb.



Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μ g/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	1:50
Chromatin IP / Chromatin IP-seq	1:25
Optimal ChIP / ChIP-seq conditions: 20 μ l of antibody & 10 μ g of chromatin (4×10^6 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) 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) Wang, H. et al. (2004) *Nature* 431, 873-8.
- (7) Kim, H. et al. (2009) *Nucleic Acids Res* 37, 2940-50.
- (8) Kalb, R. et al. (2014) *Nat Struct Mol Biol* 21, 569-71.

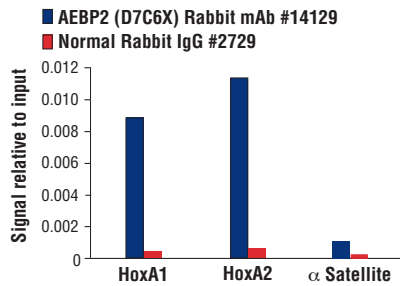
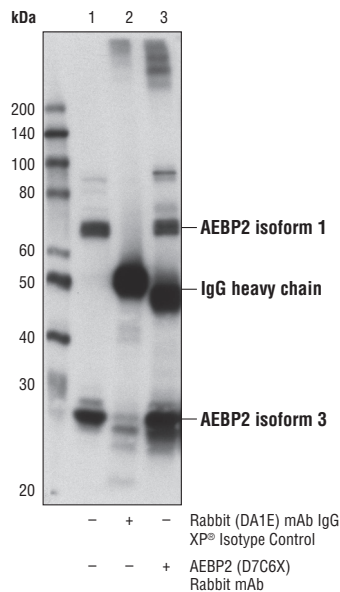
Illumina is a registered trademark of Illumina, Inc.
Tween is a registered trademark of ICI Americas, Inc.

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.

© 2014 Cell Signaling Technology, Inc.
SimpleChIP®, SignalSilence®, XP® and Cell Signaling Technology® are trademarks of Cell Signaling Technology, Inc.

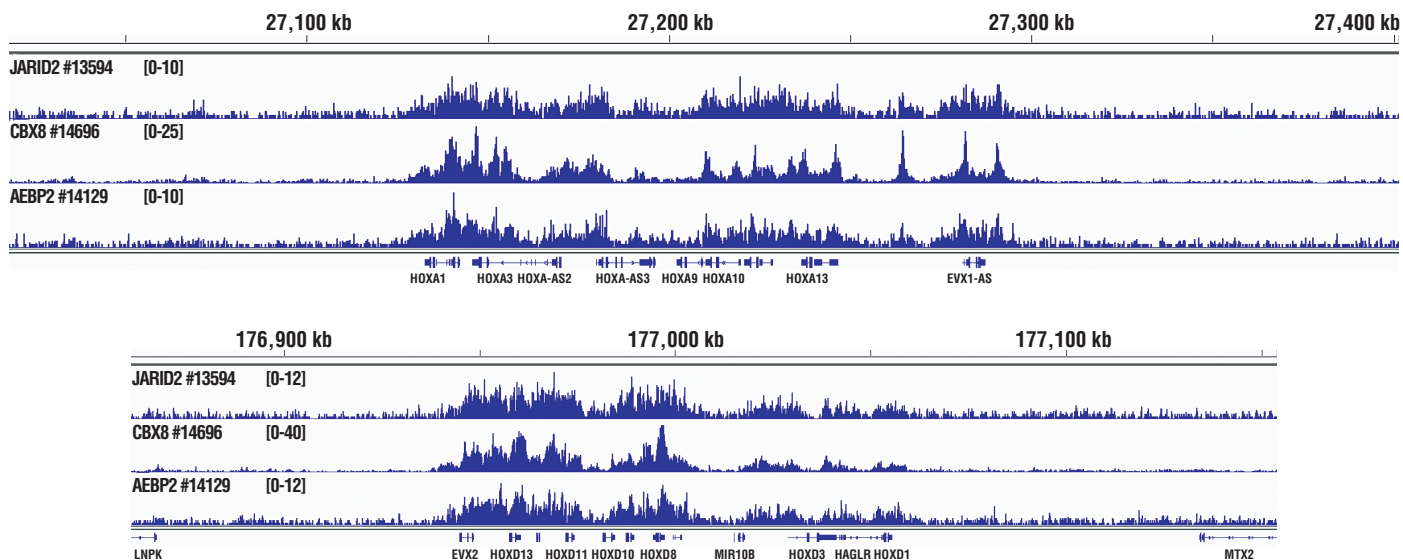
Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide Species Cross-Reactivity: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine 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.

Cell Signaling
TECHNOLOGY®



Chromatin immunoprecipitations were performed with cross-linked chromatin from 4×10^6 NCCIT cells and either 20 μ l of AEBP2 (D7C6X) Rabbit mAb or 2 μ l 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.

Immunoprecipitation of AEBP2 from NCCIT cell extracts using Rabbit (DA1E) mAb IgG XP[®] Isotype Control #3900 (lane 2) or AEBP2 (D7C6X) Rabbit mAb (lane 3). Lane 1 is 10% input. Western blot analysis was performed using AEBP2 (D7C6X) Rabbit mAb.



Chromatin immunoprecipitations were performed with cross-linked chromatin from 4×10^6 NCCIT cells and either JARID2 (D6M9X) Rabbit mAb #13594, CBX8 (D2O8C) Rabbit mAb #14696, or AEBP2 (D7C6X) 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. All of JARID2, CBX8, and AEBP2 are known to associate with PRC complexes. The figure shows binding of JARID2, CBX8, and AEBP2 across HOXA (upper) and HOXD (lower), known target genes of JARID2, CBX8, and AEBP2 (see additional figure containing ChIP-qPCR data).