

CHD4 (D4B7) Rabbit mAb



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

rev. 10/29/18

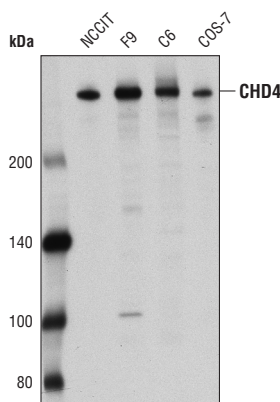
For Research Use Only. Not For Use In Diagnostic Procedures.

Applications W, IF-IC, ChIP, ChIP-seq Endogenous	Species Cross-Reactivity* H, M, R, Mk, (Hm, B, Pg, Hr)	Molecular Wt. 260 kDa	Isotype Rabbit IgG**
--	--	--------------------------	-------------------------

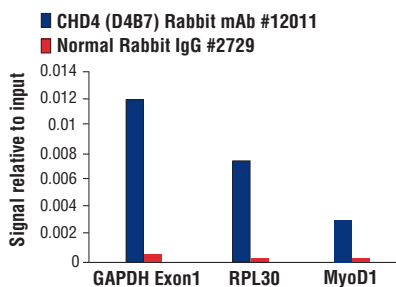
Background: Chromodomain-helicase-DNA-binding domain (CHD) proteins have been identified in a variety of organisms (1,2). This family of nine proteins is divided into three separate subfamilies: subfamily I (CHD1 and CHD2), subfamily II (CHD3 and CHD4), and subfamily III (CHD5, CHD6, CHD7, CHD8, CHD9). All CHD proteins contain two tandem amino-terminal chromodomains, a SWI/SNF-related ATPase domain, and a carboxy-terminal DNA-binding domain (1,2). The chromodomains facilitate binding to methylated lysine residues of histone proteins and confer interactions with specific regions of chromatin. The SWI/SNF-related ATPase domain utilizes energy from ATP hydrolysis to modify chromatin structure. CHD proteins are often found in large, multiprotein complexes with their transcriptional activation or repression activity governed by other proteins within the complex. CHD3 (also known as Mi2- α) and CHD4 (also known as Mi2- β) are central components of the nucleosome remodeling and histone deacetylase (NuRD) transcriptional repressor complex, which also contains HDAC1, HDAC2, RBAP48, RBAP46, MTA1, MTA2, MTA3, and MBD3 (3-8). Both CHD3 and CHD4 contain two plant homeodomain (PHD) zinc finger domains that bind directly to HDAC1 and HDAC2.

Specificity/Sensitivity: CHD4 (D4B7) Rabbit mAb recognizes endogenous levels of total CHD4 protein. Based on sequence alignment, this antibody is not predicted to cross-react with other CHD proteins.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Pro1533 of human CHD4 protein.



Western blot analysis of extracts from various cell lines using CHD4 (D4B7) Rabbit mAb.



Chromatin immunoprecipitations were performed with cross-linked chromatin from K562 cells and either CHD4 (D4B7) Rabbit mAb or 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 GAPDH Exon 1 Primers #5516, SimpleChIP® Human RPL30 Exon 3 Primers #7014, and SimpleChIP® Human MyoD1 Exon 1 Primers #4490. 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.

Entrez-Gene ID #1108
UniProt ID #Q14839

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:

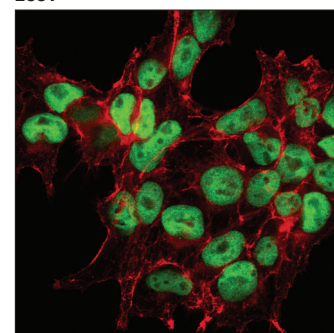
Western blotting	1:1000
Immunofluorescence (IF-IC)	1:400
Chromatin IP / Chromatin IP-seq	1:50

Optimal ChIP / ChIP-seq conditions: 10 µl of antibody & 10 µg of chromatin (4 x 10⁶ cells) per IP. Antibody validated using SimpleChIP® Enzymatic ChIP Kits

For product specific protocols please see the web page for this product at www.cellsignaling.com.

Please visit www.cellsignaling.com for a complete listing of recommended companion products.

293T

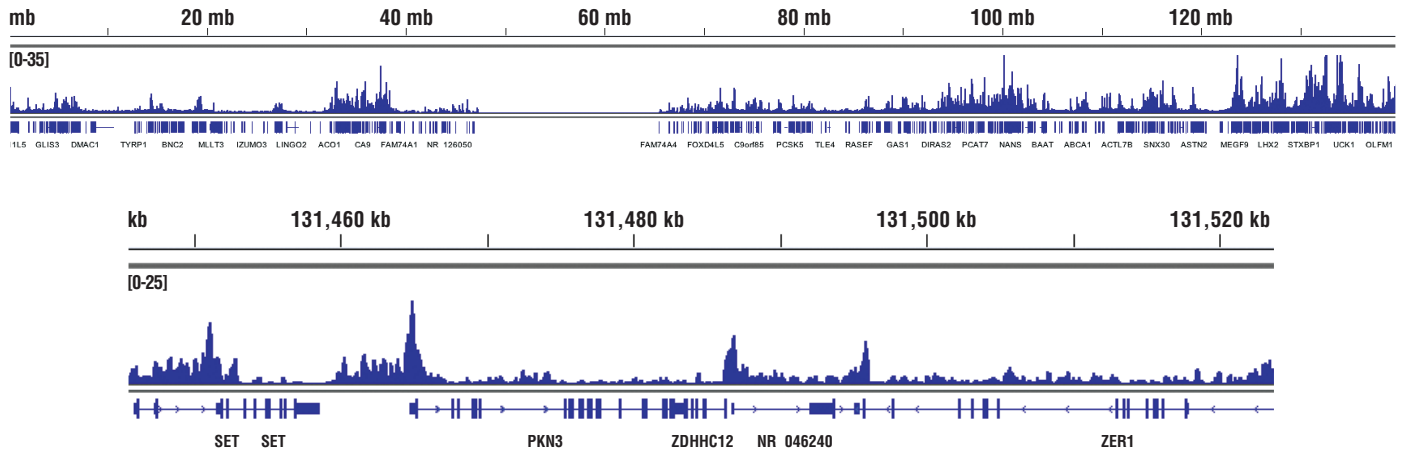


Confocal immunofluorescent analysis of 293T cells using CHD4 (D4B7) Rabbit mAb (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red).

Illumina is a registered trademark of Illumina, Inc.
DyLight is a trademark of Thermo Fisher Scientific Inc. and its subsidiaries.
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.

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 AI—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



Chromatin immunoprecipitations were performed with cross-linked chromatin from K562 cells and CHD4 (D4B7) 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 9 (upper), including PKN3 gene (lower).

Background References:

- (1) Hall, J.A. and Georgel, P.T. (2007) *Biochem Cell Biol* 85, 463-76.
- (2) Marfella, C.G. and Imbalzano, A.N. (2007) *Mutat Res* 618, 30-40.
- (3) Tong, J.K. et al. (1998) *Nature* 395, 917-21.
- (4) Xue, Y. et al. (1998) *Mol Cell* 2, 851-61.
- (5) Zhang, Y. et al. (1998) *Cell* 95, 279-89.
- (6) Bowen, N.J. et al. (2004) *Biochim Biophys Acta* 1677, 52-7.
- (7) Jones, P.L. et al. (1998) *Nat Genet* 19, 187-91.
- (8) Fujita, N. et al. (2003) *Cell* 113, 207-19.