

CTCF (D31H2) XP[®] Rabbit mAb

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Applications: W, IP, IHC-P, IF-F, IF-IC, ChIP, ChIP-seq, C&R, C&T	Reactivity: H M R Mk	Sensitivity: Endogenous	MW (kDa): 140	Source/Isotype: Rabbit IgG	UniProt ID: #P49711	Entrez-Gene Id: 10664
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Product Usage Information

For optimal ChIP or ChIP-seq results, use 10 µl of antibody and 10 µg of chromatin (approximately 4 x 10⁶ cells) per IP. This antibody has been validated using SimpleChIP[®] Enzymatic Chromatin IP Kits.

The CUT&RUN dilution was determined using CUT&RUN Assay Kit #86652.

The CUT&Tag dilution was determined using CUT&Tag Assay Kit #77552.

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:50
Immunohistochemistry (Paraffin)	1:640 - 1:2560
Immunofluorescence (Frozen)	1:200 - 1:800
Immunofluorescence (Immunocytochemistry)	1:400 - 1:1600
Chromatin IP	1:50
Chromatin IP-seq	1:50
CUT&RUN	1:50
CUT&Tag	1:50

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.

For a carrier free (BSA and azide free) version of this product see product #48859.

Specificity/Sensitivity

CTCF (D31H2) XP[®] Rabbit mAb detects endogenous levels of total CTCF protein. This antibody does not cross-react with BORIS. This antibody nonspecifically labels the lamina propria of small intestine in fixed frozen mouse tissue by immunofluorescence.

Species predicted to react based on 100% sequence homology

Bovine, Horse

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to the carboxy terminus of the human CTCF protein.

Background

CCCTC-binding factor (CTCF) and its paralog, the Brother of the Regulator of Imprinted Sites (BORIS), are highly conserved transcription factors that regulate transcriptional activation and repression, insulator function, and imprinting control regions (ICRs) (1-4). Although they have divergent amino and carboxy termini, both proteins contain 11 conserved zinc finger domains that work in combination to bind the same DNA elements (1). CTCF is ubiquitously expressed and contributes to transcriptional regulation of cell-growth regulated genes, including *c-Myc*, *p19/ARF*, *p16/INK4A*, *BRCA1*, *p53*, *p27*, *E2F1*, and *TERT* (1). CTCF also binds to and is required for the enhancer-blocking activity of all known insulator elements and ICRs, including the H19/Igf2, Prader-Willi/Angelman syndrome, and Inactive X-Specific Transcript (XIST) anti-sense loci (5-7). CTCF DNA-binding is sensitive to DNA methylation, a mark that determines selection of the imprinted allele (maternal vs. paternal) (1). The various functions of CTCF are regulated by at least two different post-translational modifications. Poly(ADP-ribosyl)ation of CTCF is required for insulator function (8). Phosphorylation of Ser612 by protein kinase CK2 facilitates a switch of CTCF from a transcriptional repressor to an activator at the *c-Myc* promoter (9). *CTCF* mutations or deletions have been found in many breast, prostate, and Wilms' tumors (10,11). Expression of BORIS is restricted to spermatocytes and is mutually exclusive of CTCF (3). In cells expressing BORIS, promoters of X-linked cancer-testis antigens like *MAGE-A1* are demethylated and activated, but methylated and inactive in CTCF-expressing somatic cells (12). Like other testis specific

proteins, BORIS is abnormally expressed in different cancers, such as breast cancer, and has a greater affinity than CTCF for DNA-binding sites, detracting from CTCF's potential tumor suppressing activity (1,3,13,14).

Background References

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7. Chao, W. et al. (2002) *Science* 295, 345-7.
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Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

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

Applications Key

W: Western Blotting **IP:** Immunoprecipitation **IHC-P:** Immunohistochemistry (Paraffin) **IF-F:** Immunofluorescence (Frozen) **IF-IC:** Immunofluorescence (Immunocytochemistry) **ChIP:** Chromatin IP **ChIP-seq:** Chromatin IP-seq **C&R:** CUT&RUN **C&T:** CUT&Tag

Cross-Reactivity Key

H: Human **M:** Mouse **R:** Rat **Mk:** Monkey

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