

Anti-CTCF

Polyclonal Antibody

Cat. # 07-729

Lot # 2475698

pack size: 200 µL

Store at -20°C

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS



Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, ChIP	H	N/A	a.a. 659-675	Rb	120 kDa	AAB07788

Background

CTCF is a ubiquitous 11 zinc finger (ZF) protein with highly versatile functions. A transcriptional repressor, CTCF, binds to promoters of vertebrate c-myc gene. It also binds to the PLK and PIM1 promoters. CTCF may prevent the access of transcriptional activators to enhancers. CTCF also acts as a transcriptional activator of APP. It is involved in different aspects of gene regulation including promoter activation or repression, hormone-responsive gene silencing, methylation-dependent chromatin insulation, and genomic imprinting. CTCF may also act as tumor suppressor. CTCF organizes epigenetically controlled chromatin insulators that regulate imprinted genes in soma.

Presentation

Rabbit polyclonal antiserum containing 0.05% sodium azide and 30% glycerol.

Specificity

Recognizes CTCF at a.a. 659-675.

Species Cross-reactivity

Human. Predicted to cross-react with mouse, rat, dog and primates based on sequence homology.

Immunogen

KLH-conjugated, synthetic peptide corresponding to amino acids 659-675 (C-TNQPKNQPTAIQVED) of human CCCTC-binding factor (CTCF) with a N-terminal cysteine added for conjugation purposes.

Molecular Weight

120 kDa

Storage and Handling

Stable for 1 year at -20°C from date of receipt.

Handling Recommendations: Upon first thaw, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C.

Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance. Note: Variability in freezer temperatures below -20°C may cause glycerol containing solutions to become frozen during storage.

Control

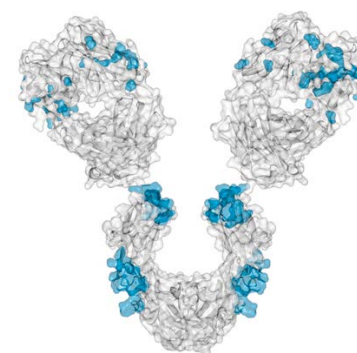
HeLa nuclear extract.

Included Positive Antigen Control: Catalog # 12-309, HeLa nuclear extract. Add an equal volume of Laemmli reducing sample buffer to 10 µL of extract and boil for 5 minutes to reduce the preparation. Load 20 µg of reduced extract per lane for minigels.

Quality Control Testing

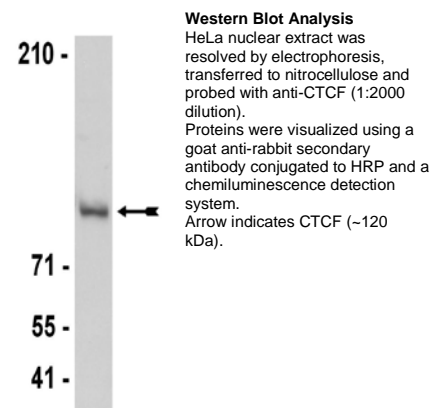
Routinely evaluated by western blot.

Western Blot Analysis: A 1:1000–1:5000 dilution of this lot detected CTCF in HeLa nuclear extract. A previous lot detected CTCF in K562 nuclear extract (data not shown).



References

1. Delgado, M.D., *et al.* (1999). *FEBS Lett.* 444: 5-10.
2. Filippova, G.N., *et al.* (1998). *Genes Chromosomes Cancer.* 22: 26-36.
3. Klenova, E.M., *et al.* (1998). *J. Biol. Chem.* 273: 26571-26579.
4. Filippova, G.N., *et al.* (1996). *Mol. Cell Biol.* 16: 2802-2813.



APPLICATION LEGEND: WB Western Blotting ChIP Chromatin Immunoprecipitation IP Immunoprecipitation

IC Immunocytochemistry IH Immunohistochemistry (Tissue)

SPECIES LEGEND: H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates

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Additional Research Applications

Chromatin Immunoprecipitation: An independent laboratory has confirmed that this antibody immunoprecipitates CTCF-bound chromatin from WEHI B-cells.

PROTOCOL

Western Blot

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a HeLa nuclear extract sample (using a modified protocol of Dignam, et al., *Nucleic Acids Res.* **22:** 1475, 1983) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared TBS containing 3% nonfat dry milk (Catalog # 20-200) and 0.05% Tween®-20 (TBST-MLK) for 1 hour at room temperature with constant agitation.
3. Incubate the nitrocellulose with **1:1000-1:5000 dilution of anti-CTCF**, diluted in freshly prepared TBSTMLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, Catalog # 12-348, 1:5000 dilution was used) in TBST-MLK for 90 minutes with agitation at room temperature.
6. Wash the nitrocellulose twice with water.
7. Wash the nitrocellulose in TBS-0.05% Tween®-20 for 10 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

RELATED PRODUCTS (specific)

cat #	description
12-309	■ HeLa Nuclear Extract
12-348	■ Goat Anti-Rabbit IgG

RELATED PRODUCTS (non-specific)

cat #	description
IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 um
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 um
IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 um
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0050	■ IMMOBILON WESTERN CHEMILUM HRP SUBSTRATE 50 mL
17-373SP	■ Spray & Glow™ ECL Western Blotting 40 mL
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G
2170	■ CHEMIBLOCKER-1LT
20-200	■ IMMUNOBLOT BLOCKING REAGENT 20G
12-302	■ EGF-Stimulated A431 Cell Lysate
12-349	■ Goat Anti-Mouse IgG, HRP conjugate
12-110	■ Phosphotyrosine control (EGF-stim A431 cell lysate)

■ antibodies ■ Multiplex products ■ biotools ■ cell culture ■ enzymes ■ kits ■ proteins/peptides ■ siRNA/cDNA products

Please visit www.millipore.com for additional product information, test data and references

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