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Datasheet for 600-401-907

BORIS Antibody

Overview

Description:	Anti-BORIS (RABBIT) Antibody - 600-401-907
Item No.:	600-401-907
Size:	100 µg
Applications:	ELISA, WB, ChIP, IF, Multiplex
Reactivity:	Human
Host Species:	Rabbit

Product Details

Background:	Anti-BORIS antibody is designed, produced, and validated as part of a collaboration between Rockland and the National Cancer Institute (NCI) and is suitable for Cancer, Immunology and Nuclear Signaling research. BORIS (Brother of the Regulator of Imprinted Sites) also known as CCCTC-binding factor-like protein, is normally only expressed in the testis and expressed in a mutually exclusive manner with CTCF during male germ cell development. However, previous studies have shown that BORIS is abnormally activated in a wide range of human cancers. Expression of BORIS in normally BORIS-negative cells promotes cell growth that may lead to transformation. BORIS maps to the cancer-associated amplification region thought to contain an oncogene or dominant-immortalizing gene. BORIS is a candidate protein for the epigenetic reprogramming factor acting in the male germ line. BORIS is found in both the nucleus and cytoplasm. BORIS antibody can be used to investigate epigenetic regulation.
Synonyms:	rabbit anti-BORIS Antibody, Transcriptional repressor CTCFL, Brother of the regulator of imprinted sites antibody, CCCTC binding factor (zinc finger protein) like antibody, CCCTC-binding factor antibody, CTCF paralog antibody, CTCF-like, cancer/testis antigen 27, CT27, CTCF-T
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	CTCFL
Reactivity:	Human

Immunogen Type:	Conjugated Peptide
Immunogen:	This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to N-Terminal region near aa 1-30 of human BORIS protein.
Purity/Specificity:	This affinity-purified antibody is directed against the human BORIS protein. The product was affinity purified from monospecific antiserum by immunoaffinity purification. A BLAST analysis was used to suggest cross reactivity with BORIS proteins from human and chimpanzee. Partial reactivity may occur against BORIS from mouse, dog and rat sources based on varying degrees of homology to the immunizing sequence. Reactivity against homologues from other sources is not known.
Relevant Links:	• UniProtKB - Q8NI51 • NCBI - 20805280 • GeneID - 140690

Application Details

Tested Applications:	ELISA, WB
Suggested Applications:	ChIP, IF, Multiplex (Based on references)
Application Note:	BORIS affinity purified antibody has been tested for use in ELISA and by western blot. Specific conditions for reactivity should be optimized by the end user. Expect a predominant band approximately 75 kDa in size corresponding to BORIS by western blotting in the appropriate cell lysate or extract.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:2,000 - 1:10,000
WB:	1:200 - 1:2,000

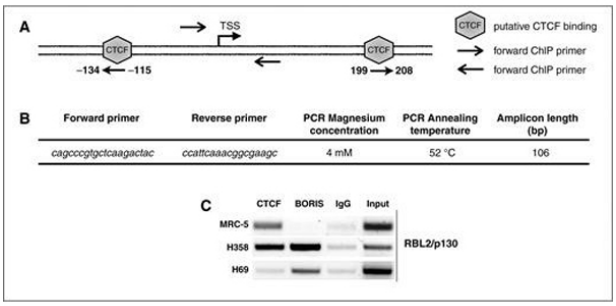
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.28 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

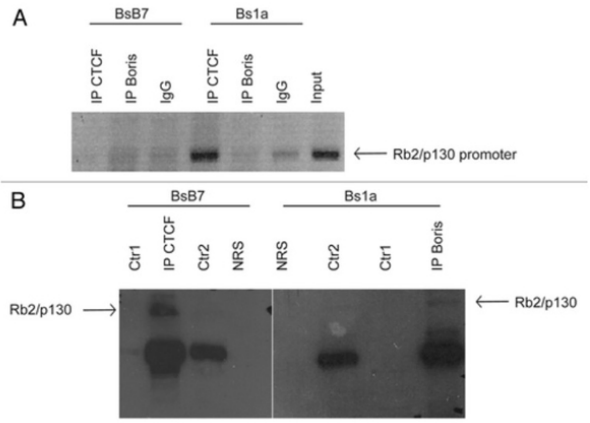
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store BORIS Antibody at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images

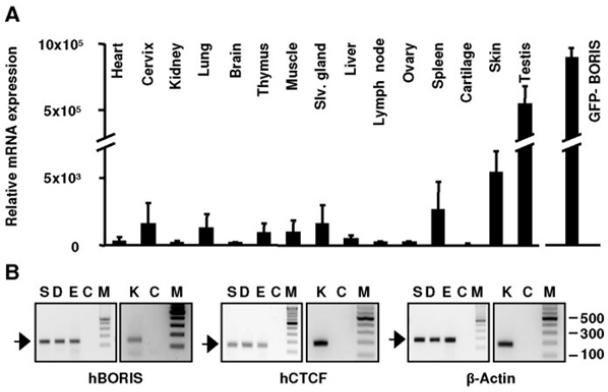


Immunoprecipitation
A, schematic representation of Rb2/p130 gene region containing CTCF putative binding sites. B, nucleotide sequence of the primers spanning the Rb2/p130 region investigated. C, in vivo binding (XChIP) of CTCF and Boris to Rb2/p130 (RBL2/P130) promoter in MRC-5 lung fibroblasts, and H358 (NCSCL) and H69 (SCLC)lung cancer cell lines. The XChIPs were performed using the ChIP-IT Express Enzymatic kit (Active Motif). For each immunoprecipitation, 10 µg of sheared chromatin was incubated overnight with 2 µg of polyclonal antibody specific for CTCF (Millipore, DAM1421463), BORIS (Rockland, 600–401-907) or normal rabbit IgG (Santa Cruz, sc-2027). PCR was then performed on 1/10 of immunoprecipitated DNA using specific primers for Rb2/p130 core promoter region (B). As positive control for CTCF and BORIS DNA–protein binding, PCR was performed amplyfing the H19/DMR locus (Nguyen, 2008; ref. 21). Input represents the 0.25% of total preimmunoprecipitated chromatin. The results were confirmed by three independent experiments. Fig 1. PMID: 21325284



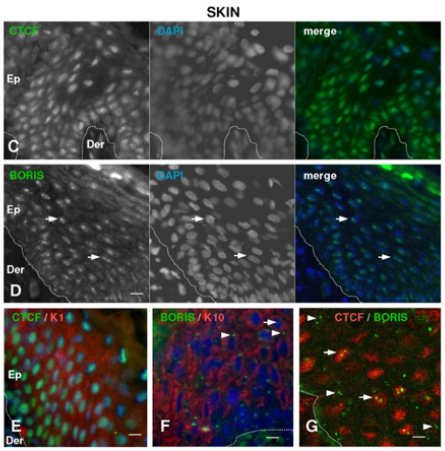
ChIP

A) In vivo binding (XChIP) of CTCF and BORIS to Rb2/p130 promoter in BsB7 (T-Ag-positive) and Bs1a (T-Ag-negative) mouse medulloblastoma cell lines. For each immunoprecipitation, 10 µg of sheared chromatin was incubated overnight with 2 µg of polyclonal antibody specific for CTCF (Millipore, DAM1421463), BORIS (Rockland, 600-401-907) or normal rabbit IgG (Santa Cruz, sc-2027). PCR was then performed on 1/10 of immunoprecipitated DNA using specific primers for Rb2/p130 core promoter region (Fiorentino et al., 2011). As positive control for CTCF and CTCFL/BORIS DNA-protein binding, PCR was performed by amplifying the H19/DMR locus (21). Input represents 0.25% of total preimmunoprecipitated chromatin. The results were confirmed by three independent experiments;
B) Rb2/p130 co-immunoprecipitates with CTCF and BORIS in mouse medulloblastoma BsB7 (T-Ag-positive) cells. Immunoprecipitation experiments were performed from total fractions and using rabbit polyclonal anti-CTCF or anti-BORIS as immunoprecipitating antibodies. The presence of Rb2/p130 in both CTCF and BORIS precipitates was assessed with mouse monoclonal anti-Rb2/p130 antibody by western blotting. Ctr1 (Supernatant+Antibody) and Ctr2 (Beads +Antibody) were used as immunoprecipitation controls. Figure 2. PMID: 22544282

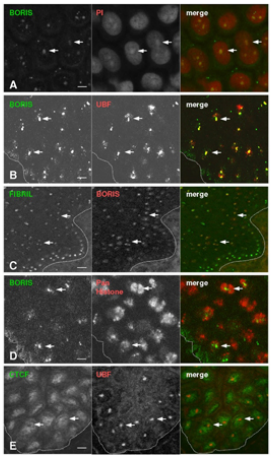


Western Blot

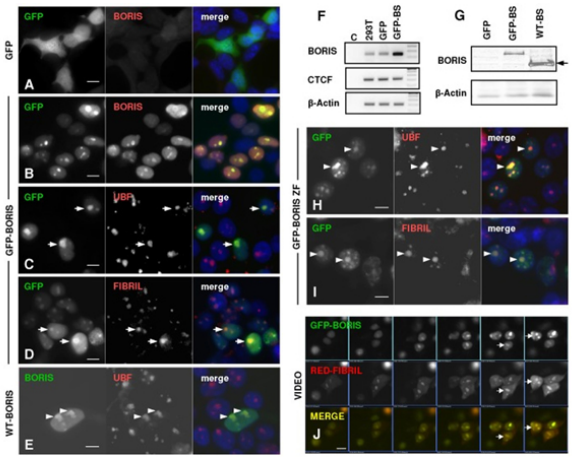
Expression of BORIS and CTCF in mouse tissues and human skin.
A) BORIS mRNA expression in mouse tissues as analysed by quantitative RT-PCR by the comparative Ct method and normalised to GAPDH. Data are represented as fold changes relative to the lowest BORIS/GAPDH ratio (cartilage, designated as 1.0). For each sample, measurements were done in duplicate using two different primer sets. Error bars represent s.d. HEK293T cells transfected with pEGFP-mBORIS were used as positive control (right graph). B) BORIS, CTCF and β-Actin (internal control) mRNA expression in human skin and primary keratinocytes by semiquantitative RT-PCR (H0.3 primer set was used, see Information S1). Human total skin (S), dermis (D), epidermis (E) and freshly isolated keratinocytes (K), buffer only-control (C) or molecular weight markers (M). Figure 1. PMID: 22724006



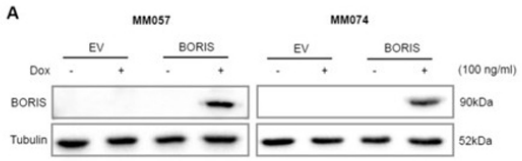
Immunofluorescence Microscopy
Expression of BORIS and CTCF in human skin.
C,D) Indirect immunofluorescence experiments on human skin sections with anti-CTCF or anti-BORIS antibodies as indicated. Colours as indicated. The nuclei were visualised with DAPI (blue). Dotted line indicates the basal membrane that separates the epidermis (Ep) from the dermis (Der). Scale bar: 50 µm. Photographs are representative of studies on five different human individuals with three different polyclonal antibodies for BORIS and two different antibodies for CTCF. E,F) Double immunofluorescence for CTCF or BORIS and markers of post-mitotic terminal differentiation keratins K1/K10. Colours as indicated. Scale bar: 20 µm. G) Double immunofluorescence for CTCF (red) or BORIS (green). Scale bar: 10 µm. Arrows indicate the focal accumulation of BORIS within the nuclei, arrowheads indicate BORIS dots beside the nuclei. Colours as indicated. Figure 1. PMID: 22724006



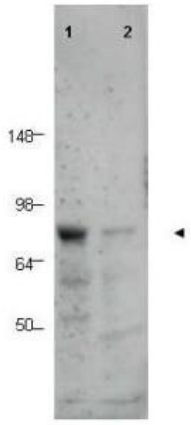
Immunofluorescence Microscopy
BORIS localises to the nucleoli of human epidermal cells. A–E). Double immunofluorescence analyses were performed on human skin sections with antibodies to BORIS or CTCF and UBF, Fibrillarin or pan-histone, as indicated. Coulors as indicated. Nucleoli were counterstained with propidium iodie (PI) in A. Arrows point at nucleoli (A–C,E) or histone-dark areas (D). Note the coincidence between the BORIS protein, nucleolar markers and dark-histone areas. Scale bar: 20 µm. Fig 2. PMID: 22724006



Immunofluorescence Microscopy
Exogenous GFP-BORIS localises to the nucleoli.
A–E, H,I.
Detection of GFP (green) or BORIS (red), UBF, or Fibrillarin by immunofluorescence in HEK293T cells 24 hours after transfection with plasmids carrying GFP (A) or GFP-BORIS (B-D), wild-type BORIS (E) or GFP-Zinc finger domain of BORIS (H,I), as indicated on the left side. The nuclei were visualised with DAPI (blue). Scale bar: 10 µm. F) Detection of BORIS mRNA expression by RT-PCR in HEK293T cells transiently transfected as above. Primers for CTCF and β-actin were used as controls. BS: BORIS. G) Detection of BORIS by western blotting with the antibodies used for the immunofluorescence analyses above. Note the higher molecular weight of the fusion protein GFP-BORIS, compared to the wild-type protein (arrow). β-Actin as loading control. J) Video microphotograms showing the co-localisation of GFP-BORIS and Fibrillarin-Cherry in live cells after transient transfection (see also Video S1). Fig 3.
PMID: 22724006



Western Blot
A) Expression of BORIS fused to a triple FLAG-tag was induced in the MM057 and MM074 melanoma cell lines with 100 ng/ml dox for 5 days. Whole-cell lysate was used for immunoblotting (n = 3) with anti-BORIS antibody. Anti-Tubulin was used as a loading control. Fig 6. PMID: 32123577



Western Blot
Western blot using Rockland's Affinity Purified anti-BORIS antibody shows detection of a predominant band corresponding to BORIS in human tissue lysates (arrowhead). Lane 1 contains lysate from human prostate tissue. Lane 2 contains lysate from human spleen tissue. A predominant band at ~75 kDa is observed. Molecular weight estimation was made by comparison to prestained MW markers as indicated.

References

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