abcam

Product datasheet

Anti-SATB2 antibody [EPNCIR130A] ab92446

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-SATB2 antibody [EPNCIR130A]
Description	Rabbit monoclonal [EPNCIR130A] to SATB2
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF, Flow Cyt, IHC-Fr **Unsuitable for:** IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human SATB2. The exact sequence is proprietary. Database link: Q9UPW6
Positive control	WB: HT-1080, SW1353, MCF7 and Saos-2 cell lysates. Rat and mouse brain and human fetal brain tissue lysates. IHC-P: Human cerebral cortex tissue; Mouse brain tissue. ICC/IF: SH-SY5Y cells. Flow Cyt: SH-SY5Y cells.
General notes	This antibody was developed as part of a collaboration between Epitomics, the National Cancer Institute's Center for Cancer Research and the lab of John Niederhuber. View antibodies from NCI Center for Cancer Research Collaboration. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents. **We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.** This product is a recombinant rabbit monoclonal antibody.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide

	Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPNCIR130A
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab92446** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★★	1/1000 - 1/5000. Predicted molecular weight: 81 kDa.
IHC-P		1/150 - 1/300. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.
ICC/IF	★★★★☆	1/100.
Flow Cyt		1/1000. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-Fr	★★★★★	Use at an assay dependent concentration.

Application notes	Is unsuitable for IP.

Target

Function	Binds to DNA, at nuclear matrix- or scaffold-associated regions. Thought to recognize the sugar-phosphate structure of double-stranded DNA. Transcription factor controlling nuclear gene expression, by binding to matrix attachment regions (MARs) of DNA and inducing a local chromatin-loop remodeling. Acts as a docking site for several chromatin remodeling enzymes and also by recruiting corepressors (HDACs) or coactivators (HATs) directly to promoters and enhancers. Required for the initiation of the upper-layer neurons (UL1) specific genetic program and for the inactivation of deep-layer neurons (DL) and UL2 specific genes, probably by modulating BCL11B expression. Repressor of Ctip2 and regulatory determinant of corticocortical connections in the developing cerebral cortex. May play an important role in palate formation. Acts as a molecular node in a transcriptional network regulating skeletal development and osteoblast differentiation.
Tissue specificity	High expression in adult brain, moderate expression in fetal brain, and weak expression in adult liver, kidney, and spinal cord and in select brain regions, including amygdala, corpus callosum, caudate nucleus, and hippocampus.
Involvement in disease	Note=Chromosomal aberrations involving SATB2 are found in isolated cleft palate. Translocation t(2;7); translocation t(2;11). Defects in SATB2 are a cause of cleft palate isolated (CPI) [MIM:119540]. A congenital fissure of the soft and/or hard palate, due to faulty fusion. Isolated cleft palate is not associated with cleft lips. Some patients may manifest other craniofacial dysmorphic features, mental retardation, and

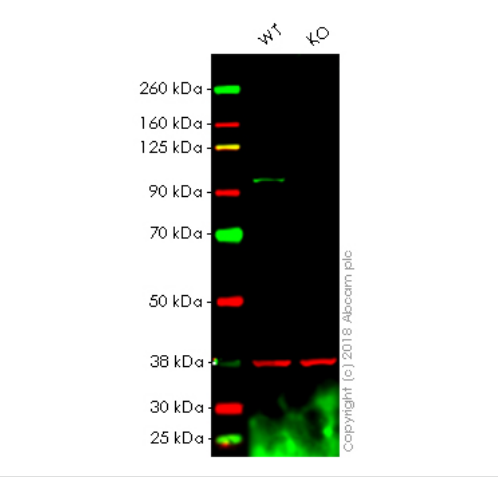
	osteoporosis. Note=A chromosomal aberration involving SATB2 is found in a patient with classical features of Toriello-Carey syndrome. Translocation t(2;14)(q33;q22).
Sequence similarities	Belongs to the CUT homeobox family. Contains 2 CUT DNA-binding domains. Contains 1 homeobox DNA-binding domain.
Post-translational modifications	Sumoylated by PIAS1. Sumoylation promotes nuclear localization, but represses transcription factor activity.
Cellular localization	Nucleus matrix.

Images

Immunohistochemistry (Frozen sections) - Anti-SATB2 antibody [EPNCIR130A] (ab92446)

Image courtesy of an anonymous Abreview.

Unpurified ab92446 staining SATB2 in mouse brain tissue by Immunohistochemistry (Frozen sections). Tissue was fixed with paraformaldehyde and permeabilized using 0.3% Triton-X-100. Samples were then blocked with 5% serum for 1 hour 30 minutes at 20°C followed by incubation with the primary antibody at a 1/200 dilution for 36 hours. A biotin conjugated goat anti-rabbit polyclonal was used as secondary antibody at a 1/200 dilution.



Western blot - Anti-SATB2 antibody [EPNCIR130A] (ab92446)

All lanes : Anti-SATB2 antibody [EPNCIR130A] (ab92446) at 1/1000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : SATB2 knockout HAP1 whole cell lysate

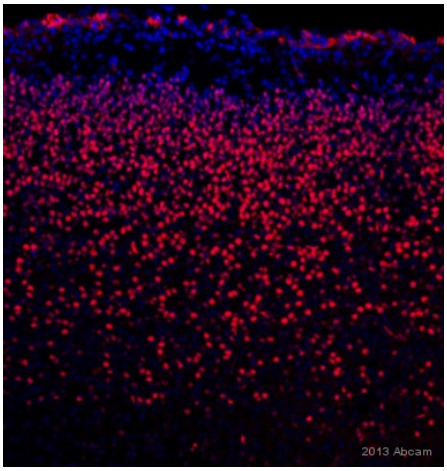
Lysates/proteins at 20 µg per lane.

Predicted band size: 81 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab92446 observed at 83 kDa. Red - loading control, ab9484, observed at 37 kDa.

ab92446 was shown to specifically react with SATB2 in wild-type HAP1 cells as signal was lost in SATB2 knockout cells. Wild-type and SATB2 knockout samples were subjected to SDS-PAGE.

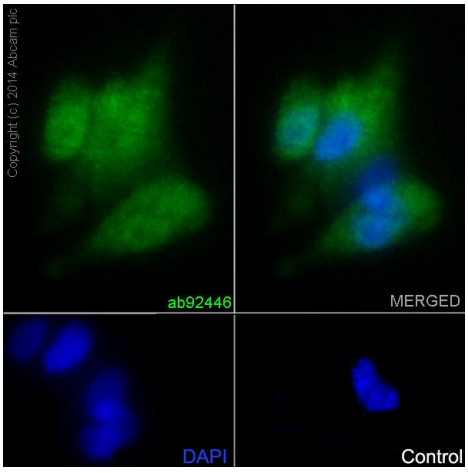
Ab92446 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SATB2 antibody [EPNCIR130A] (ab92446)

This image is courtesy of an anonymous Abreview

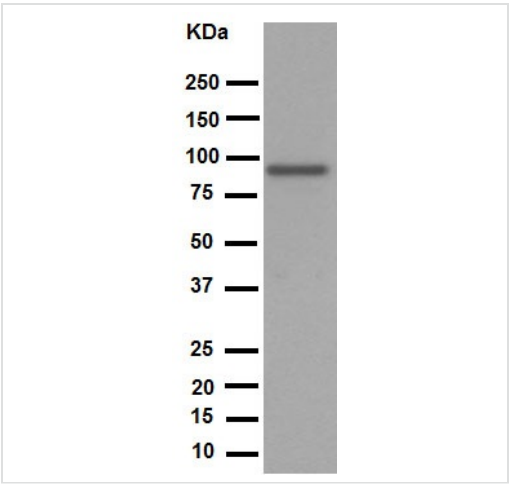
Unpurified ab92446 staining SATB2 in E18 Mouse brain tissue sections by Immunohistochemistry (PFA perfusion fixed frozen sections). Tissue samples were fixed by perfusion with paraformaldehyde, permeablized with 0.3% Triton-X 100 and blocked with 3% BSA for 30 minutes at 25°C. The sample was incubated with primary antibody (1/500 in TBS with 0.1% Triton-X 100 + 3% Goat serum) at 4°C for 12 hours. An Alexa Fluor® 546-conjugated Goat anti-rabbit polyclonal (1/1000) was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-SATB2 antibody [EPNCIR130A] (ab92446)

Immunocytochemistry/Immunofluorescence analysis of SH-SY5Y (human neuroblastoma cell line from bone marrow) cells labelling SATB2 (green) with purified ab92446 at 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain.

Control: primary antibody (1/100) and secondary antibody ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).



Western blot - Anti-SATB2 antibody [EPNCIR130A] (ab92446)

Anti-SATB2 antibody [EPNCIR130A] (ab92446) at 1/1000 dilution (purified) + SW1353 cell lysate at 20 µg

Secondary

Peroxidase conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

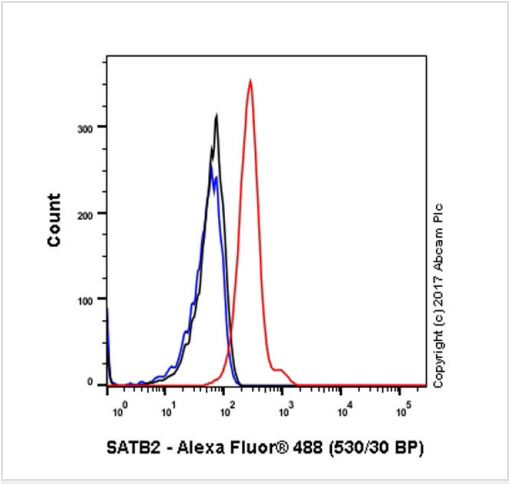
Predicted band size: 81 kDa

Observed band size: 83 kDa

why is the actual band size different from the predicted?

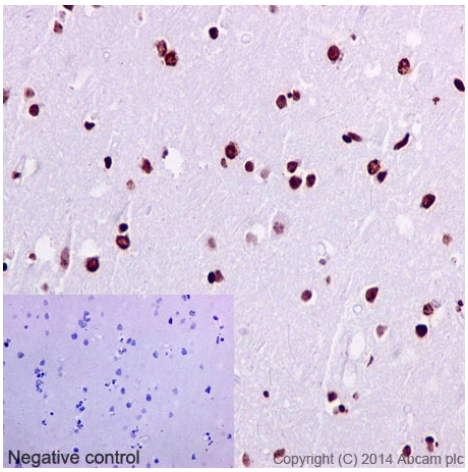
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



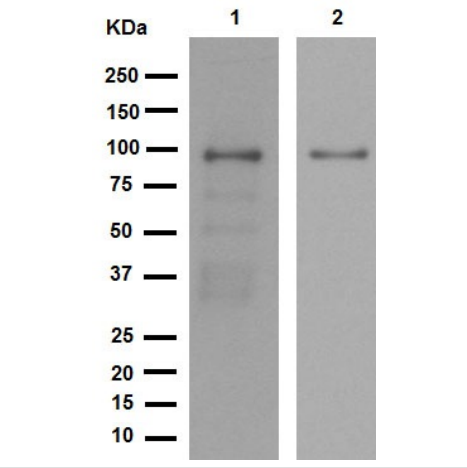
Flow Cytometry - Anti-SATB2 antibody [EPNCIR130A] (ab92446)

Flow Cytometry analysis of SH-SY5Y cells (human neuroblastoma cell line from bone marrow) labeling SATB2 with purified ab92446 at 1/150 dilution(10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488)(ab150077)(1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) (ab172730) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SATB2 antibody [EPNCIR130A] (ab92446)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cerebral cortex tissue labelling SATB2 with purified ab92446 at 1/150. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit IgG was used as the secondary antibody. Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Western blot - Anti-SATB2 antibody [EPNCIR130A] (ab92446)

All lanes : Anti-SATB2 antibody [EPNCIR130A] (ab92446) at 1/1000 dilution (purified)

Lane 1 : Saos-2 (human osteosarcoma cell line) cell lysate
Lane 2 : Human fetal brain tissue lysate

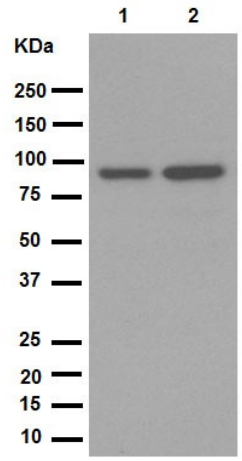
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Peroxidase conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 81 kDa
Observed band size: 83 kDa why is the actual band size different from the predicted?

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-SATB2 antibody [EPNCIR130A] (ab92446)

All lanes : Anti-SATB2 antibody [EPNCIR130A] (ab92446) at 1/1000 dilution (purified)

Lane 1 : Mouse brain tissue lysate
Lane 2 : Rat brain tissue lysate

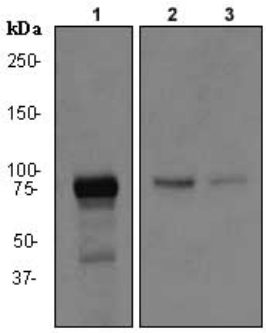
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Peroxidase conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 81 kDa
Observed band size: 83 kDa why is the actual band size different from the predicted?

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-SATB2 antibody [EPNCIR130A] (ab92446)

All lanes : Anti-SATB2 antibody [EPNCIR130A] (ab92446) at 1/1000 dilution (unpurified)

Lane 1 : HT1080 (human fibrosarcoma cell line) cell lysate
Lane 2 : SW1353 cell lysate
Lane 3 : MCF7 (human breast adenocarcinoma cell line) cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 81 kDa

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