abcam

Product datasheet

Anti-SATB2 antibody [EPNCIR130A] - BSA and Azide free ab212177

KO VALIDATED Recombinant RabMAb

8 Images

Overview

Product name	Anti-SATB2 antibody [EPNCIR130A] - BSA and Azide free
Description	Rabbit monoclonal [EPNCIR130A] to SATB2 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), IHC-P, WB, ICC/IF **Unsuitable for:** IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
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. ICC/IF: Saos-2 cells
General notes	ab212177 is the carrier-free version of **ab92446**. 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 **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply

- Animal-free production

For more information **see here**.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPNCIR130A
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab212177 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
Flow Cyt (Intra)		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
WB		Use at an assay dependent concentration. Predicted molecular weight: 81 kDa.
ICC/IF		Use at an assay dependent concentration.

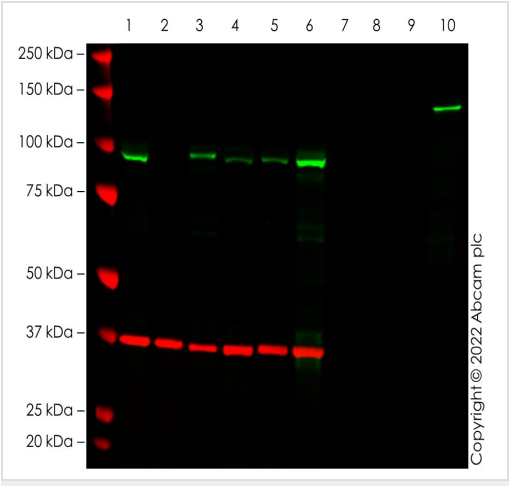
Application notes Is unsuitable for IP.

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



Western blot - Anti-SATB2 antibody [EPNCIR130A] - BSA and Azide free (ab212177)

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

Lane 1 : Wild-type HAP1 cell lysate at 20 µg
Lane 2 : SATB2 knockout HAP1 cell lysate at 20 µg
Lane 3 : Mouse E18 Embyonic brain cell lysate at 20 µg
Lane 4 : NIH/3T3 cell lysate at 20 µg
Lane 5 : HT1080 cell lysate at 20 µg
Lane 6 : Saos-2 cell lysate at 20 µg
Lanes 7 & 9 : Empty
Lane 8 : SATB1 Recombinant Protein cell lysate at 0.1 µg
Lane 10 : SATB2 Recombinant Protein (**ab132405**) cell lysate at 0.1 µg

Secondary
All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

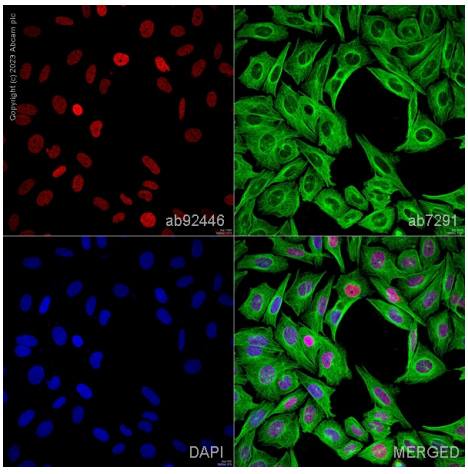
Predicted band size: 81 kDa

Observed band size: 100 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab92446**).

False colour image of Western blot: Anti-SATB2 antibody [EPNCIR130A] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab92446** was shown to bind specifically to SATB2. A band was observed at 100 kDa in wild-type HAP1 cell lysates with no signal observed at this size in SATB2 knockout cell line.

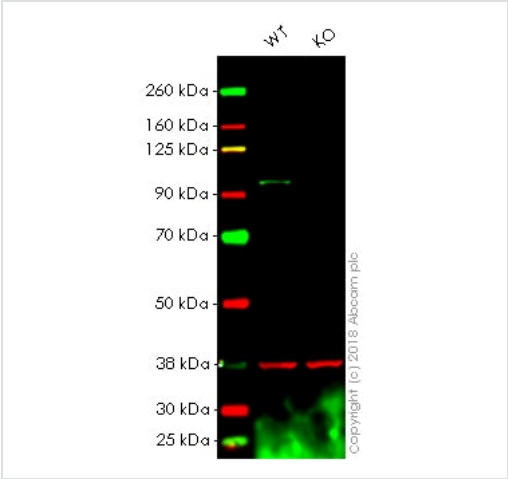
To generate this image, wild-type and SATB2 knockout HAP1 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-SATB2 antibody [EPNCIR130A] - BSA and Azide free (ab212177)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92446**).

Immunocytochemistry/Immunofluorescence analysis of Saos-2 cells (human osteosarcoma cell line) labelling SATB2 (red) with purified **ab92446** at 1/100 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150083**, an Alexa Fluor® 647-conjugated goat anti-rabbit IgG H&L (1/1000), was used as the secondary antibody. Tubulin (green) was stained with **ab7291**, an anti-alpha tubulin antibody (1/1000). DAPI (blue) was used as the nuclear counterstain.



Western blot - Anti-SATB2 antibody [EPNCIR130A] - BSA and Azide free (ab212177)

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

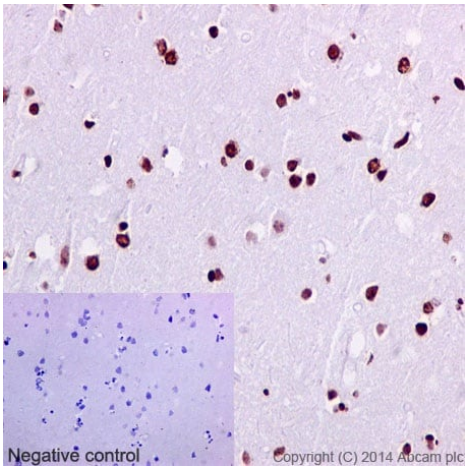
Lysates/proteins at 20 µg per lane.

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.

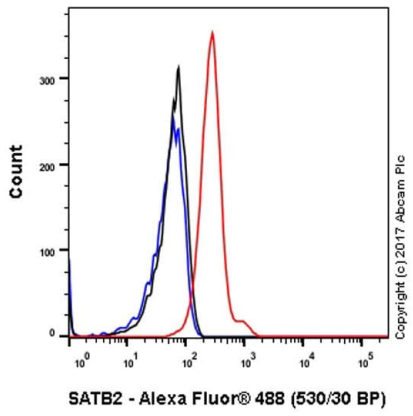
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92446**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SATB2 antibody [EPNCIR130A] - BSA and Azide free (ab212177)

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.

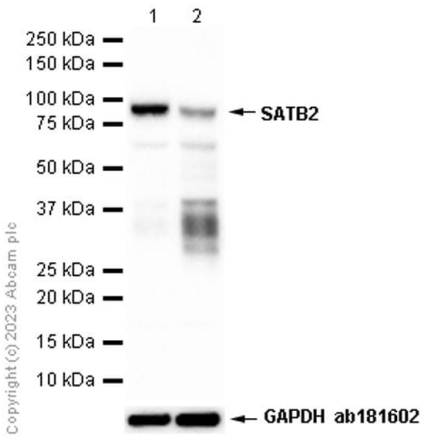
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92446**).



Flow Cytometry (Intracellular) - Anti-SATB2 antibody [EPNCIR130A] - BSA and Azide free (ab212177)

Intracellular 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92446**).



Western blot - Anti-SATB2 antibody [EPNCIR130A] - BSA and Azide free (ab212177)

All lanes : Anti-SATB2 antibody [EPNCIR130A] (**ab92446**) at 1/10000 dilution

Lane 1 : Saos-2 (Human osteosarcoma epithelial) whole cell fresh lysate
Lane 2 : Saos-2 (Human osteosarcoma epithelial) whole cell lysate

Lysates/proteins at 20 µg per lane.

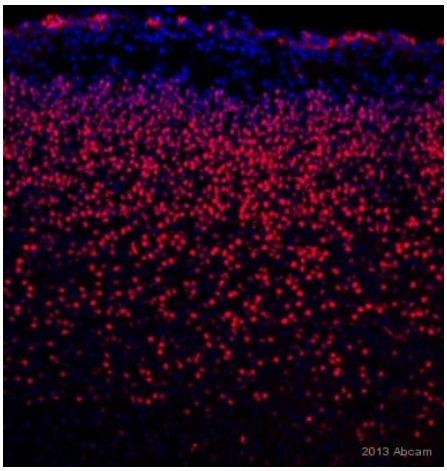
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 81 kDa
Observed band size: 83 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab92446**).

Blocking and diluting buffer and concentration: 5% NFDM/TBST

Freshly made lysate is highly recommended to minimize protein degradation.

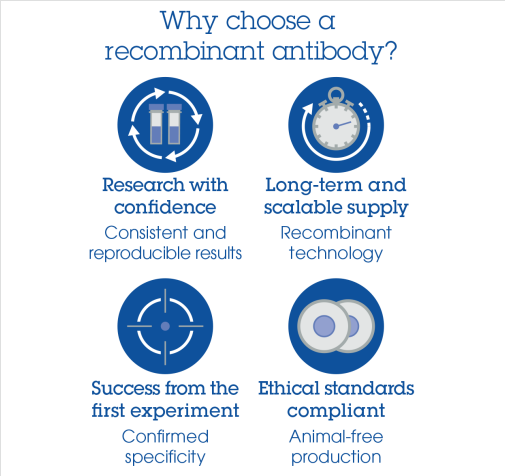


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SATB2 antibody [EPNCIR130A] - BSA and Azide free (ab212177)
This image is courtesy of an anonymous Abreview.

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92446**).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Anti-SATB2 antibody [EPNCIR130A] - BSA and Azide free (ab212177)

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