abcam

Product datasheet

Anti-SATB1 antibody [EPR3951] ab109122





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Overview

Product name Anti-SATB1 antibody [EPR3951]

Description Rabbit monoclonal [EPR3951] to SATB1

Host species Rabbit

Tested applications Suitable for: IP, ICC/IF, WB, IHC-P, Flow Cyt

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HAP1, Jurkat and THP1 whole cell lysate.

General notes 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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number **EPR3951**

Isotype ΙgG The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab109122 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 |
|-------------|-----------|---|
| IP | | 1/20. |
| ICC/IF | | 1/50. |
| WB | | 1/1000. Predicted molecular weight: 86 kDa. |
| IHC-P | **** (1) | 1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. heat up to 98 degrees C, below boiling, and then let cool for 10-20 min. |
| Flow Cyt | | 1/20. |

Target

Function

Crucial silencing factor contributing to the initiation of X inactivation mediated by Xist RNA that occurs during embryogenesis and in lymphoma (By similarity). Binds to DNA at special AT-rich sequences, the consensus SATB1-binding sequence (CSBS), at nuclear matrix- or scaffoldassociated regions. Thought to recognize the sugar-phosphate structure of double-stranded DNA. Transcriptional repressor controlling nuclear and viral gene expression in a phosphorylated and acetylated status-dependent manner, 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 (e.g. PML at the MHC-I locus) and also by recruiting corepressors (HDACs) or coactivators (HATs) directly to promoters and enhancers. Modulates genes that are essential in the maturation of the immune T-cell CD8SP from thymocytes. Required for the switching of fetal globin species, and beta- and gamma-globin genes regulation during erythroid differentiation. Plays a role in chromatin organization and nuclear architecture during apoptosis. Interacts with the unique region (UR) of cytomegalovirus (CMV). Alu-like motifs and SATB1-binding sites provide a unique chromatin context which seems preferentially targeted by the HIV-1 integration machinery. Moreover, HIV-1 Tat may overcome SATB1-mediated repression of IL2 and IL2RA (interleukin) in T-cells by binding to the same domain than HDAC1. Delineates specific epigenetic modifications at target gene loci, directly upregulating metastasis-associated genes while downregulating tumor-suppressor genes. Reprograms chromatin organization and the transcription profiles of breast tumors to promote growth and metastasis.

Tissue specificity

 $\hbox{Expressed predominantly in thymus.} \\$

Sequence similarities

Belongs to the CUT homeobox family.

Contains 2 CUT DNA-binding domains.

Contains 1 homeobox DNA-binding domain.

Post-translational modifications

Sumovlated. Sumovlation promotes cleavage by caspases.

Phosphorylated by PKC. Acetylated by PCAF. Phosphorylated form interacts with HDAC1, but unphosphorylated form interacts with PCAF. DNA binding properties are activated by phosphorylation and inactivated by acetylation. In opposition, gene expression is down-regulated by phosphorylation but up-regulated by acetylation.

Cleaved at Asp-254 by caspase-3 and caspase-6 during T-cell apoptosis in thymus and during B-cell stimulation. The cleaved forms can not dimerize and lose transcription regulation function because of impaired DNA and chromatin association.

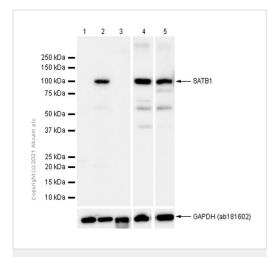
Cellular localization

Nucleus matrix. Nucleus > PML body. Organized into a cage-like network anchoring loops of heterochromatin and tethering specialized DNA sequences. When sumoylated, localized in promyelocytic leukemia nuclear bodies.

Form

There are 2 isoforms produced by alternative splicing.

Images



Western blot - Anti-SATB1 antibody [EPR3951] (ab109122)

All lanes : Anti-SATB1 antibody [EPR3951] (ab109122) at 1/1000 dilution (Purified)

Lane 1 : LNCaP (Human prostate carcinoma epithelial cell) whole cell lysate

Lane 2 : THP-1 (Human monocytic leukemia monocyte) whole cell lysate

Lane 3 : Mouse liver lysate

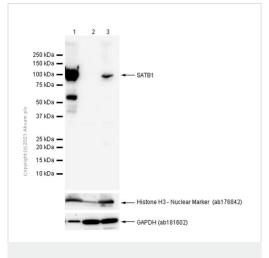
Lane 4 : Mouse thymus lysate

Lane 5 : Rat thymus lysate

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 86 kDa



Western blot - Anti-SATB1 antibody [EPR3951] (ab109122)

All lanes : Anti-SATB1 antibody [EPR3951] (ab109122) at 1/1000 dilution (Purified)

Lane 1 : Jurkat (Human T cell leukemia T lymphocyte) nuclear extract lysate

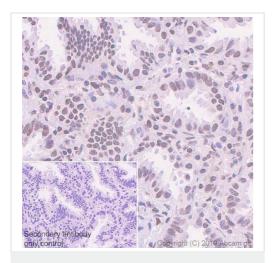
Lane 2 : Jurkat (Human T cell leukemia T lymphocyte) without nuclear extract lysate

Lane 3 : Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate

Secondary

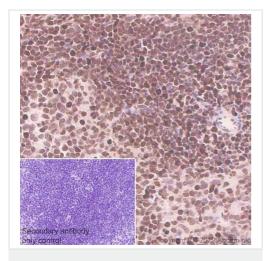
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 86 kDa



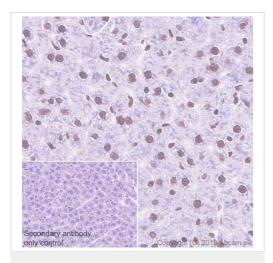
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SATB1 antibody
[EPR3951] (ab109122)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human lung carcinoma tissue sections labeling SATB1 with Purified ab109122 at 1:500 dilution (0.246 µg/ml). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) . Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



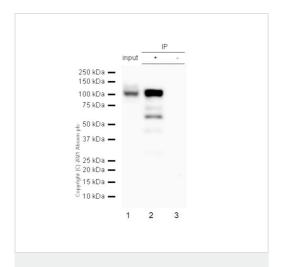
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SATB1 antibody
[EPR3951] (ab109122)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse liver tissue sections labeling SATB1 with Purified ab109122 at 1:500 dilution (0.246 µg/ml). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) . Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SATB1 antibody
[EPR3951] (ab109122)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat liver tissue sections labeling SATB1 with Purified ab109122 at 1:500 dilution (0.246 µg/ml). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



Immunoprecipitation - Anti-SATB1 antibody [EPR3951] (ab109122)

Purified ab109122 at 1:20 dilution $(0.6\mu g)$ immunoprecipitating SATB1 in Rat thymus lysate.

Lane 1 (input): Rat thymus lysate 10 µg

Lane 2 (+): ab109122 + Rat thymus lysate.

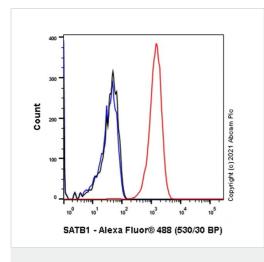
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab109122 in Rat thymus lysate.

VeriBlot for IP Detection Reagent (HRP)(<u>ab131366</u>) (1:5000 dilution) was used for Western blotting.

Blocking Buffer and concentration: 5% NFDM/TBST.

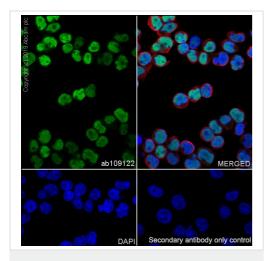
Diluting buffer and concentration: 5% NFDM/TBST.

Observed band size: kDa



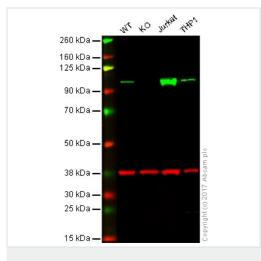
Flow Cytometry - Anti-SATB1 antibody [EPR3951] (ab109122)

Flow Cytometry analysis of Jurkat (Human T cell leukemia T lymphocyte) cells labelling SATB1 with Purified ab109122 at 1:20 dilution (10 µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit lgG (Alexa Fluor® 488, ab150077) secondary antibody was used at 1:2000. Isotype control - Rabbit monoclonal lgG (Black). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunocytochemistry/ Immunofluorescence - Anti-SATB1 antibody [EPR3951] (ab109122)

Immunocytochemistry analysis of Jurkat (Human T cell leukemia T lymphocyte) cells labeling SATB1 with Purified ab109122 at 1:50 dilution (2.5 μ g/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 μ g/ml). Goat anti rabbit lgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1:1000 (2 μ g/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Western blot - Anti-SATB1 antibody [EPR3951] (ab109122)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)

Lane 2: SATB1 knockout HAP1 whole cell lysate (20 µg)

Lane 3: Jurkat whole cell lysate (20 µg)

Lane 4: THP1 whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab109122 observed at 100 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab109122 was shown to specifically react with SATB1 in wild-type HAP1 cells as signal was lost in SATB1 knockout cells. Wild-type and SATB1 knockout samples were subjected to SDS-PAGE. Ab109122 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.



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