abcam

Product datasheet

Anti-SOX9 antibody [EPR14335-78] - BSA and Azide free ab225541

Recombinant RabMAb

1 References 13 Images

Overview

Product name Anti-SOX9 antibody [EPR14335-78] - BSA and Azide free

Description Rabbit monoclonal [EPR14335-78] to SOX9 - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), ICC/IF, WB, IHC-P

Species reactivity Reacts with: Mouse, Rat, Human

Predicted to work with: Cow, Dog, Pig, Common marmoset

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control IHC-P: Mouse eye tissue. Rat, mouse and human colon tissue. Human breast carcinoma tissue.

WB: SW480 and PC-3 cell lysate. ICC/IF: SW480, F9 and PC-3 cells, primary hippocampal

mouse neurons/glia DIV14. Flow Cyt (intra): PC-3 cells.

General notes ab225541 is the carrier-free version of <u>ab185966</u>.

Our <u>carrier-free</u> 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.

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

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Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal
Clone number EPR14335-78

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab225541 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. ab199376 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 70 kDa (predicted molecular weight: 56 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer.

rarget

Function Plays an important role in the normal skeletal development. May regulate the expression of other

genes involved in chondrogenesis by acting as a transcription factor for these genes.

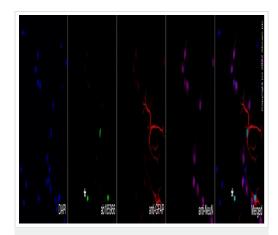
Involvement in disease Defects in SOX9 are the cause of campomelic dysplasia (CMD1) [MIM:114290]. CMD1 is a rare,

often lethal, dominantly inherited, congenital osteochondrodysplasia, associated with male-to-female autosomal sex reversal in two-thirds of the affected karyotypic males. A disease of the newborn characterized by congenital bowing and angulation of long bones, unusually small scapulae, deformed pelvis and spine and a missing pair of ribs. Craniofacial defects such as cleft palate, micrognatia, flat face and hypertelorism are common. Various defects of the ear are often evident, affecting the cochlea, malleus incus, stapes and tympanum. Most patients die soon after birth due to respiratory distress which has been attributed to hypoplasia of the tracheobronchial

cartilage and small thoracic cage.

Sequence similarities Contains 1 HMG box DNA-binding domain.

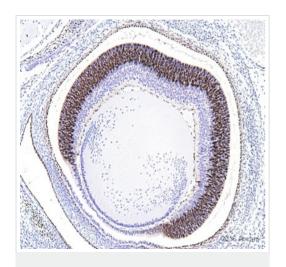
Cellular localization Nucleus.



Immunocytochemistry/ Immunofluorescence - Anti-SOX9 antibody [EPR14335-78] - BSA and Azide free (ab225541)

Immunofluorescence staining of SOX9 using <u>ab185966</u> in primary hippocampal mouse neurons/glia, (obtained from Transnetyx Tissue by BrainBits, LLC, cat.no. C57EHP), DIV14. The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% TritonX-100 (in PBS) for 5 mins and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with <u>ab185966</u> at 1 μg/ml, <u>ab4674</u> (anti-GFAP) at 1/1000 dilution and <u>ab104224</u> (anti-NeuN) at 1/1000 dilution. Cells were then incubated with <u>ab150081</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) preadsorbed at 1/1000 dilution (shown in green), <u>ab150176</u>, Goat Anti-Chicken lgY H&L (Alexa Fluor[®] 594) preadsorbed (shown in red) and <u>ab150119</u>, Goat Anti-Mouse lgG H&L (Alexa Fluor[®] 647) (shown in purple), all secondary antibodies at 1/1000 dilution. Nuclear DNA was labelled with DAPI (shown in blue).

As expected, most GFAP positive cells are also SOX9 positive, while NeuN positive cells are SOX9 negative. SOX9 positive cells, which are not GFAP positive (e.g. asterisk) are likely neural stem cells/ oligodendrocyte precursor cells present in the culture. Images were acquired with the Perkin Elmer Operetta HCA and a maximum intensity projection of confocal sections is shown. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185966).

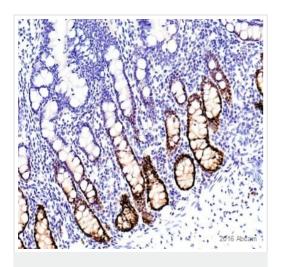


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SOX9 antibody

[EPR14335-78] - BSA and Azide free (ab225541)

This image is courtesy of an abreview submitted by Carl Hobbs, King's College London, United Kingdom

ab185966 staining SOX9 in developing eye of mouse tissue section by Immunohistochemistry (PFA perfusion fixed frozen sections). Tissue samples were fixed by perfusion with formaldehyde, cut into 20 micron slices, blocked with 2% BSA for 10 minutes at 21°C and antigen retrieval was by heat mediation in citrate buffer. The sample was incubated with primary antibody (1/1000 in PBS) at 21°C for 4 hours. A Biotin-conjugated goat antirabbit polyclonal (1/300) was used as the secondary antibody.



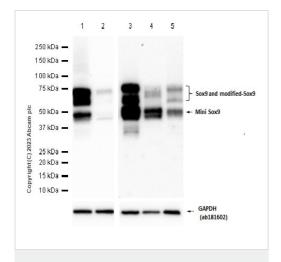
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SOX9 antibody

[EPR14335-78] - BSA and Azide free (ab225541)
This image is courtesy of an abreview submitted by Carl Hobbs, King's College London, United Kingdom

Immunohistochemical analysis of Formalin/PFA-fixed paraffinembedded pig small intestine sections labelling SOX9 with **ab185966** at dilution of 1/2000. The secondary antibody used was a polyclonal goat anti-rabbit biotin conjugated antibody at a dilution of 1/300. The sample was counterstained with hematoxylin. Antigen retrieval was heat mediated using citric acid.

The image shows intense enterocyte/goblet cell nuclear positivity, confined to the crypts of Lieberkühn.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185966).



Western blot - Anti-SOX9 antibody [EPR14335-78] - BSA and Azide free (ab225541)

All lanes : Anti-SOX9 antibody [EPR14335-78] (**ab185966**) at 1/1000 dilution

Lane 1 : SW480 (Human colorectal adenocarcinoma epithelial cell) whole cell lysate

Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 3: NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate

Lane 4 : Mouse colon tissue lysate

Lane 5 : Mouse P0 bone tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 56 kDa

Observed band size: 42,56,75 kDa

Exposure time: 180 seconds

Blocking and diluting buffer: 5% NFDMTBST

SOX9 can be ubiquitinated or SUMOylated to higher molecular weight (PMID: 24155239, PMID: 16307912, PMID: 16554309, PMID: 32070068). Meanwhile, it has a truncated version as mini-Sox9 (PMID: 21297661,PMID: 27429045).

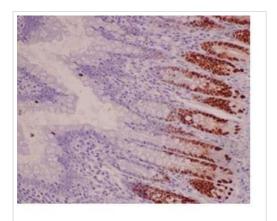
HeLa expresses very low level of SOX9 (PMID: 18296708, PMID: 18677406).

SOX9 - Alexa Fluor® 488 (530/30 BP)

Flow Cytometry (Intracellular) - Anti-SOX9 antibody [EPR14335-78] - BSA and Azide free (ab225541)

Intracellular Flow Cytometry analysis of PC-3 (human prostate adenocarcinoma) cells labeling SOX9 with purified <u>ab185966</u> at 1/120 (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (<u>ab150077</u>) (1/2000 dilution) was used as the secondary antibody. Rabbit IgG, monoclonal [EPR25A] - Isotype Control (<u>ab172730</u>) (Black) 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 (ab185966).

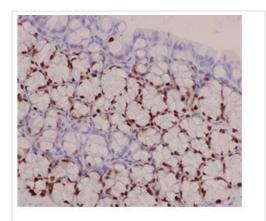


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SOX9 antibody

[EPR14335-78] - BSA and Azide free (ab225541)

Immunohistochemistry analysis of paraffin-embedded Rat colon tissue labeling SOX9 with <u>ab185966</u> at 1/1000 dilution.

Counterstained with Hematoxylin.

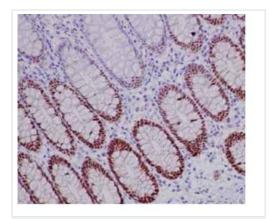


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SOX9 antibody
[EPR14335-78] - BSA and Azide free (ab225541)

Immunohistochemistry analysis of paraffin-embedded Mouse colon tissue labeling SOX9 with <u>ab185966</u> at 1/1000 dilution.

Counterstained with Hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185966).



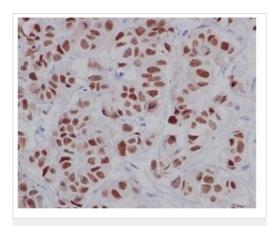
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SOX9 antibody

[EPR14335-78] - BSA and Azide free (ab225541)

Immunohistochemistry analysis of paraffin-embedded Human colon tissue labeling SOX9 with <u>ab185966</u> at 1/1000 dilution.

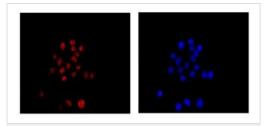
Counterstained with Hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185966).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SOX9 antibody
[EPR14335-78] - BSA and Azide free (ab225541)

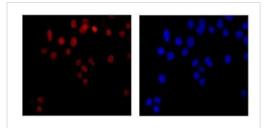
Immunohistochemistry analysis of paraffin-embedded Human breast carcinoma tissue labeling SOX9 with <u>ab185966</u> at 1/1000 dilution. Counterstained with Hematoxylin.



Immunocytochemistry/ Immunofluorescence - Anti-SOX9 antibody [EPR14335-78] - BSA and Azide free (ab225541)

Immunofluorescence analysis of 4% paraformaldehyde fixed SW480 cells labeling SOX9 with **ab185966** at 1/250 dilution. Goat anti Rabbit lgG (Alexa Fluor®555) used as secondary antibody at 1/200 dilution. Dapi staining shown in blue.

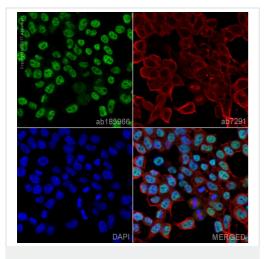
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185966).



Immunocytochemistry/ Immunofluorescence - Anti-SOX9 antibody [EPR14335-78] - BSA and Azide free (ab225541)

Immunofluorescence analysis of 4% paraformaldehyde fixed PC3 cells labeling SOX9 with <u>ab185966</u> at 1/250 dilution. Goat anti Rabbit lgG (Alexa Fluor®555) used as secondary antibody at 1/200 dilution. Dapi staining shown in blue.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185966).

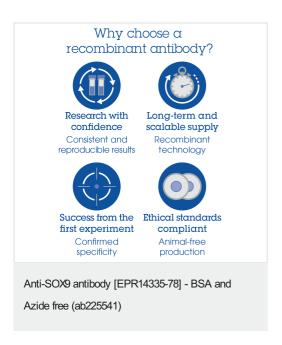


Immunocytochemistry/ Immunofluorescence - Anti-SOX9 antibody [EPR14335-78] - BSA and Azide free (ab225541)

This ICC data was generated using the same anti-SOX9 antibody clone [EPR14335-78] in a different buffer formulation (cat# ab185966).

ab185966 staining Sox9 in F9 cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab185966 at a 5μg/ml concentration and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin, at 1μg/ml concentration, followed by a further incubation at room temperature for 1h with an anti-rabbit AlexaFluor® 488 (ab150081) at 2 μg/ml (shown in green) and an anti-mouse AlexaFluor® 594 (ab150120) at 2 μg/ml (shown in pseudocolor red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



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