# abcam

# Product datasheet

# Anti-SOX9 antibody [EPR14335-78] ab185966

Recombinant RabMAb

★★★★★ 26 Abreviews 175 References 13 Images

Overview

**Product name** Anti-SOX9 antibody [EPR14335-78]

**Description** Rabbit monoclonal [EPR14335-78] to SOX9

**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

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

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** 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** 

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

**Purity** Protein A purified

Clonality Monoclonal

Clone number EPR14335-78

**Isotype** IgG

#### **Applications**

#### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab185966 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. <b>ab172730</b> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
ICC/IF	<b>★★★★★</b> (5)	Use a concentration of 1 - 5 μg/ml.
WB	<b>★★★★ (4)</b>	1/1000 - 1/10000. Detects a band of approximately 70 kDa (predicted molecular weight: 56 kDa).
IHC-P	**** <u>(6)</u>	1/1000. Perform heat mediated antigen retrieval with citrate buffer.

#### **Target**

**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.

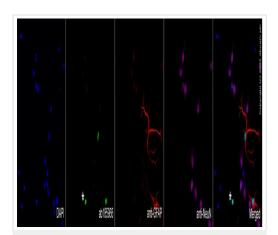
Sequence similarities

Contains 1 HMG box DNA-binding domain.

Cellular localization

Nucleus.

# **Images**



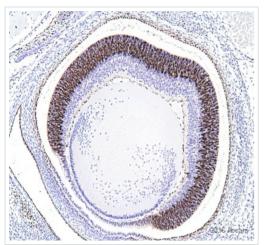
Immunocytochemistry/ Immunofluorescence - Anti-SOX9 antibody [EPR14335-78] (ab185966)

Immunofluorescence staining of SOX9 using ab185966 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 ab185966 at 1 µg/ml, ab4674 (anti-GFAP) at 1/1000 dilution and ab104224 (anti-NeuN) at 1/1000 dilution. Cells were then incubated with ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) preadsorbed at 1/1000 dilution (shown in green), ab150176, Goat Anti-Chicken IgY H&L (Alexa Fluor<sup>®</sup> 594) preadsorbed (shown in red) and ab150119, Goat Anti-Mouse IgG H&L (Alexa Fluor<sup>®</sup> 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.

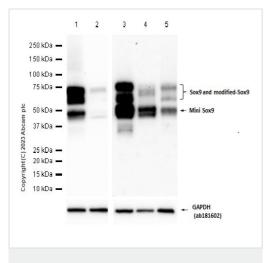


Tissue samples were fixed by perfusion with formaldehyde, cut into 20 micron slices, blocked with 2% BSA for 10 minutes at 21°C and antigen retrieval was by heat mediation in citrate buffer. The sample was incubated with primary antibody (1/1000 in PBS) at 21°C for 4 hours. A biotin-conjugated goat anti-rabbit polyclonal (1/300) was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SOX9 antibody
[EPR14335-78] (ab185966)

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



Western blot - Anti-SOX9 antibody [EPR14335-78] (ab185966)

**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

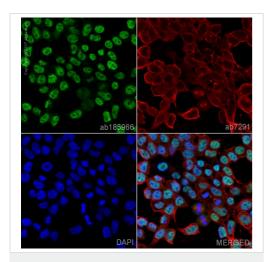
Observed band size: 42,56,75 kDa

Exposure time: 180 seconds

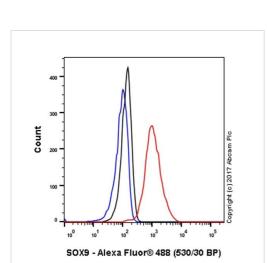
Blocking and diluting buffer: 5% NFDMTBST

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).



Immunocytochemistry/ Immunofluorescence - Anti-SOX9 antibody [EPR14335-78] (ab185966)



Flow Cytometry (Intracellular) - Anti-SOX9 antibody [EPR14335-78] (ab185966)

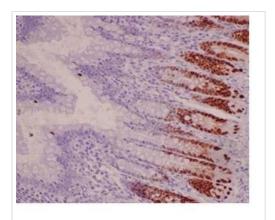
ab185966 staining Sox9 in F9 (Mouse embryonic testicular cancer cell line) cells.

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 5  $\mu$ g/ml concentration and **ab7291**, mouse monoclonal [DM1A] to alpha Tubulin, at 1 $\mu$ g/ml concentration, followed by a further incubation at room temperature for 1 h with an anti-rabbit AlexaFluor® 488 (**ab150081**) at 2  $\mu$ g/ml (shown in green) and an anti-mouse AlexaFluor® 594 (**ab150120**) at 2  $\mu$ g/ml (shown in pseudocolor red). Nuclear DNA was labeled with DAPI (shown in blue).

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

Intracellular Flow Cytometry analysis of PC-3 (human prostate adenocarcinoma) cells labeling SOX9 with purified ab185966 at 1/120 (red).

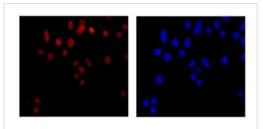
Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) (1/2000 dilution) was used as the secondary antibody. Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.



Immunohistochemistry analysis of paraffin-embedded rat colon tissue labeling SOX9 with ab185966 at 1/1000 dilution.

Counterstained with hematoxylin.

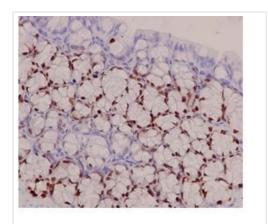
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SOX9 antibody
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Immunocytochemistry/ Immunofluorescence - Anti-SOX9 antibody [EPR14335-78] (ab185966)

Immunofluorescence analysis of 4% paraformaldehyde fixed PC-3 (Human prostate adenocarcinoma cell line) cells labeling SOX9 with ab185966 at 1/250 dilution (Left panel, red).

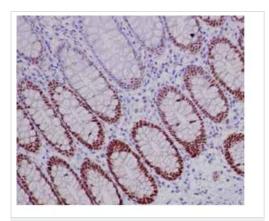
Goat anti Rabbit lgG (Alexa Fluor®555) used as secondary antibody at 1/200 dilution. DAPI staining (Right panel, blue).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SOX9 antibody
[EPR14335-78] (ab185966)

Immunohistochemistry analysis of paraffin-embedded mouse colon tissue labeling SOX9 with ab185966 at 1/1000 dilution.

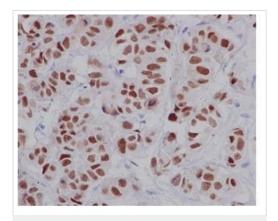
Counterstained with hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SOX9 antibody
[EPR14335-78] (ab185966)

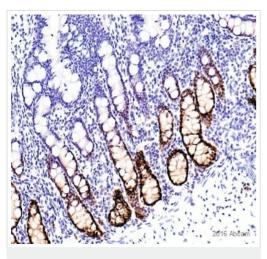
Immunohistochemistry analysis of paraffin-embedded human colon tissue labeling SOX9 with ab185966 at 1/1000 dilution.

Counterstained with hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SOX9 antibody
[EPR14335-78] (ab185966)

Immunohistochemistry analysis of paraffin-embedded human breast carcinoma tissue labeling SOX9 with ab185966 at 1/1000 dilution. Counterstained with hematoxylin.



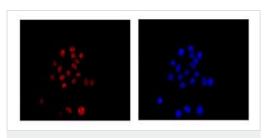
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SOX9 antibody
[EPR14335-78] (ab185966)

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

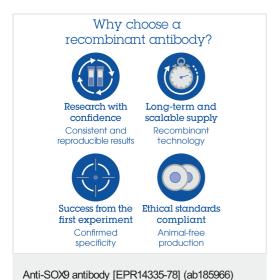
Immunohistochemical analysis of Formalin/PFA-fixed paraffinembedded pig small intestine sections labeling SOX9 with ab185966 at dilution of 1/2000.

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.



Immunocytochemistry/ Immunofluorescence - Anti-SOX9 antibody [EPR14335-78] (ab185966) Immunofluorescence analysis of 4% paraformaldehyde fixed SW480 (Human colorectal adenocarcinoma cell line) cells labeling SOX9 with ab185966 at 1/250 dilution. Goat anti Rabbit lgG (Alexa Fluor<sup>®</sup>555) used as secondary antibody at 1/200 dilution. DAPI staining shown in blue.



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