# abcam

## Product datasheet

## Anti-SOX9 antibody [EPR14335] ab185230

Recombinant RabMAb

★★★★★ 9 Abreviews 115 References 9 Images

#### Overview

**Immunogen** 

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

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

**Host species** Rabbit

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

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.

Positive control WB: SW480, HeLa and NIH/3T3 cell lysates; Mouse colon and P0 bone tissue lysates IHC-P:

Human, mouse and rat colon tissue. ICC/IF: SW480 cells and primary hippocampal mouse

neurons/glia DIV14. Flow Cyt (intra): PC3 cells. IP: SW480 cell lysate.

This product is a recombinant monoclonal antibody, which offers several advantages including: **General notes** 

- 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: 59% PBS, 40% Glycerol, 0.05% BSA

**Purity** Protein A purified

Clonality Monoclonal

Clone number EPR14335

**Isotype** IgG

### **Applications**

## The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab185230 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  |
|------------------|-----------------|--|
| ICC/IF           | **** <u>(1)</u> | Use a concentration of 5 μg/ml.  |
| WB               | <b>★★★★</b>     | 1/1000. Detects a band of approximately 70 kDa (predicted molecular weight: 56 kDa).                                       |
| IHC-P            |                 | 1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |
| IP               |                 | 1/60.  |
| Flow Cyt (Intra) |                 | 1/190.  ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.                    |

## **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.

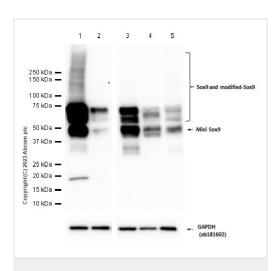
Sequence similarities

Contains 1 HMG box DNA-binding domain.

**Cellular localization** 

Nucleus.

## **Images**



Western blot - Anti-SOX9 antibody [EPR14335] (ab185230)

**All lanes :** Anti-SOX9 antibody [EPR14335] (ab185230) 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

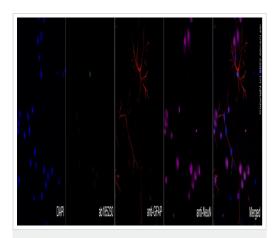
Observed band size: 42, 56, 75 kDa

Exposure time: 40 seconds

Blocking/Diluting buffer and concentration: 5% NFDM/TBST.

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).

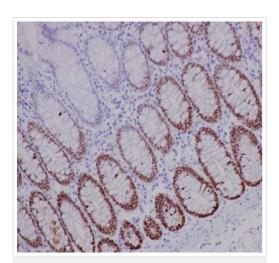


Immunocytochemistry/ Immunofluorescence - Anti-SOX9 antibody [EPR14335] (ab185230)

Immunofluorescence staining of SOX9 using ab185230 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 ab185230 at 5 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 lgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green), ab150176, Goat Anti-Chicken lgY H&L (Alexa Fluor® 594) preadsorbed (shown in red) and ab150119, 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).

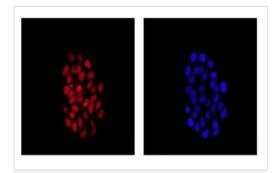
As expected, most GFAP positive cells are also SOX9 positive, while NeuN positive cells are SOX9 negative.

Images were acquired with the Perkin Elmer Operetta HCA and a maximum intensity projection of confocal sections is shown.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SOX9 antibody
[EPR14335] (ab185230)

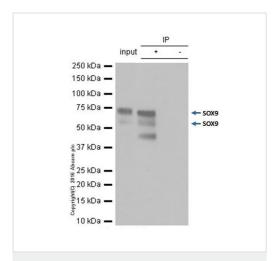
Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling SOX9 with ab185230 at 1/2000 dilution followed by pre-diluted HRP-conjugated secondary antibody and counterstained with Hematoxylin. Heat mediated antigen retrieval was performed with EDTA buffer pH 9 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-SOX9 antibody [EPR14335] (ab185230)

Immunofluorescent analysis of SW480 cells (4% paraformaldehydfixed) labeling SOX9 with ab185230 at 1/250 dilution followed by Goat anti rabbit lgG (Alexa Fluor<sup>®</sup> 555) secondary at 1/200 dilution and counter-stained with DAPI (blue).

Alexa Fluor<sup>®</sup> 488 (<u>ab196450</u>) and Alexa Fluor<sup>®</sup> 647 (<u>ab196184</u>) conjugated versions are available for this clone.



Immunoprecipitation - Anti-SOX9 antibody [EPR14335] (ab185230)

ab185230 at 1/60 immunoprecipitating SOX9 in SW480 (human colorectal adenocarcinoma) whole cell lysate observed at 56 and 75 KDa (lanes 1 and 2).

The expression pattern observed is consistent with the literature (PMID: 10682837) with an additional off-target band at 46 kD.

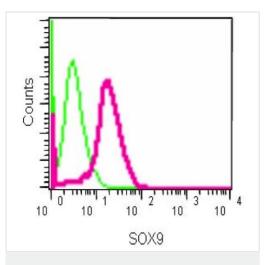
Lane 1 (input): SW480 whole cell lysate 10µg

Lane 2 (+): SW480 whole cell lysate.

**Lane 3 (-):** Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab185230 in SW480 whole cell lysate

For western blotting, ab185230 (1/1000) was used as the primary antibody and VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/10,000 dilution.

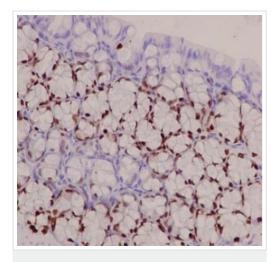
Blocking buffer and concentration: 5% NFDM/TBST. Diluting buffer and concentration: 5% NFDM /TBST.



Flow Cytometry (Intracellular) - Anti-SOX9 antibody [EPR14335] (ab185230)

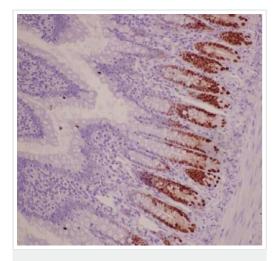
Intracellular flow cytometric analysis of PC3 cells (2% paraformaldehyde-fixed)labeling SOX9 with ab185230 at 1/190 dilution (red) or a rabbit lgG (negative) (green), followed by Goat anti rabbit lgG (FITC) secondary at 1/150 dilution.

Alexa Fluor $^{\text{@}}488$  ( $\underline{ab196450}$ ) and Alexa Fluor $^{\text{@}}647$  ( $\underline{ab196184}$ ) conjugated versions are available for this clone.



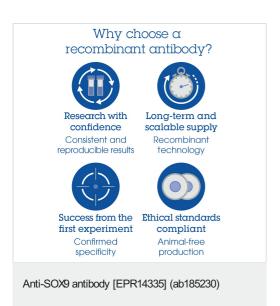
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SOX9 antibody
[EPR14335] (ab185230)

Immunohistochemical analysis of paraffin-embedded Mouse colon tissue labeling SOX9 with ab185230 at 1/2000 dilution followed by pre-diluted HRP-conjugated secondary antibody and counterstained with Hematoxylin. Heat mediated antigen retrieval was performed with EDTA buffer pH 9 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SOX9 antibody
[EPR14335] (ab185230)

Immunohistochemical analysis of paraffin-embedded Rat colon tissue labeling SOX9 with ab185230 at 1/2000 dilution followed by pre-diluted HRP-conjugated secondary antibody and counterstained with Hematoxylin. Heat mediated antigen retrieval was performed with EDTA buffer pH 9 before commencing with IHC staining protocol.



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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