abcam

Product datasheet

Anti-SOX9 antibody [EPR14335] - BSA and Azide free ab220812



2 References 9 Images

Overview

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

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

Host species Rabbit

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

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 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.

General notes ab220812 is the carrier-free version of <u>ab185230</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.

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.

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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: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal
Clone number EPR14335

Isotype IgG

Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab220812 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.
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 Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

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.

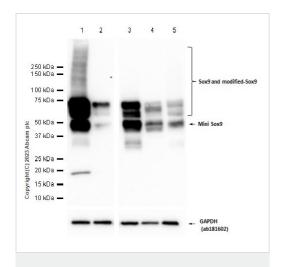
Sequence similarities

Contains 1 HMG box DNA-binding domain.

Cellular localization

Nucleus.

Images



Western blot - Anti-SOX9 antibody [EPR14335] - BSA and Azide free (ab220812)

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

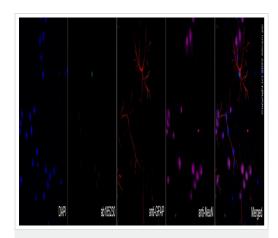
Exposure time: 40 seconds

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185230).

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).



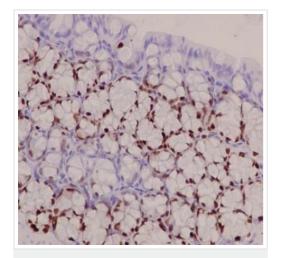
Immunocytochemistry/ Immunofluorescence - Anti-SOX9 antibody [EPR14335] - BSA and Azide free (ab220812)

Immunofluorescence staining of SOX9 using <u>ab185230</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>ab185230</u> at 5 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.

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 (ab185230).

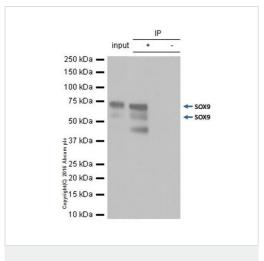


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SOX9 antibody
[EPR14335] - BSA and Azide free (ab220812)

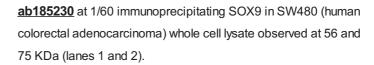
Immunohistochemical analysis of paraffin-embedded Mouse colon tissue labeling SOX9 with <u>ab185230</u> at 1/2000 dilution followed by pre-diluted HRP-conjugated secondary antibody and counterstained with Hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185230).

Perform heat mediated antigen retrieval with EDTA buffer pH 9 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-SOX9 antibody
[EPR14335] - BSA and Azide free (ab220812)



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

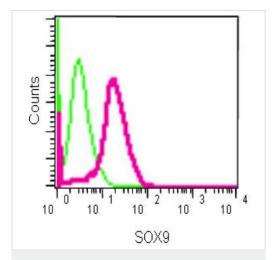
Lane 2 (+): SW480 whole cell lysate.

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

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

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

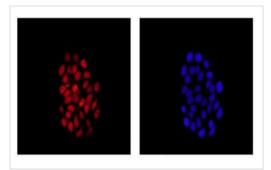
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab185230</u>).



Flow Cytometry (Intracellular) - Anti-SOX9 antibody [EPR14335] - BSA and Azide free (ab220812)

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

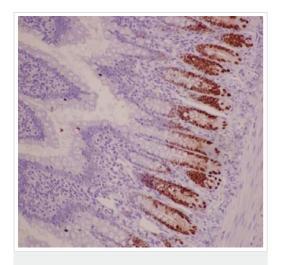
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185230).



Immunocytochemistry/ Immunofluorescence - Anti-SOX9 antibody [EPR14335] - BSA and Azide free (ab220812)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185230).



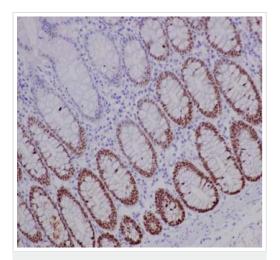
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SOX9 antibody

[EPR14335] - BSA and Azide free (ab220812)

Immunohistochemical analysis of paraffin-embedded Rat colon tissue labeling SOX9 with <u>ab185230</u> at 1/2000 dilution followed by pre-diluted HRP-conjugated secondary antibody and counterstained with Hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185230).

Perform heat mediated antigen retrieval with EDTA buffer pH 9 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SOX9 antibody [EPR14335] - BSA and Azide free (ab220812)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185230).

Perform heat mediated antigen retrieval with EDTA buffer pH 9 before commencing with IHC staining protocol.





reproducible results



technology

Anti-SOX9 antibody [EPR14335] - BSA and Azide free (ab220812)

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