abcam

ab220812

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

Recombinant | RabMAb

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR14335
Purification technique	Affinity purification Protein A
Concentration	0.977 - 0.994 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	-

Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Common marmoset, Cow, Dog, Pig
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Common marmoset, Cow, Dog, Pig
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Mouse, Human
Dilution info	-
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Common marmoset, Cow, Dog, Pig
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Common marmoset, Cow, Dog, Pig
Dilution info	-

Notes	-

IHC-P

Tested

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

Predicted

Species	Common marmoset, Cow, Dog, Pig
Dilution info	-
Notes	-

Target data

See full target information Transcription factor SOX-9

Function

Transcription factor that plays a key role in chondrocytes differentiation and skeletal development (PubMed:24038782). Specifically binds the 5'-ACAAAG-3' DNA motif present in enhancers and super-enhancers and promotes expression of genes important for chondrogenesis, including cartilage matrix protein-coding genes COL2A1, COL4A2, COL9A1, COL11A2 and ACAN, SOX5 and SOX6 (PubMed:8640233). Also binds to some promoter regions (By similarity). Plays a central role in successive steps of chondrocyte differentiation (By similarity). Absolutely required for precartilaginous condensation, the first step in chondrogenesis during which skeletal progenitors differentiate into prechondrocytes (By similarity). Together with SOX5 and SOX6, required for overt chondrogenesis when condensed prechondrocytes differentiate into early stage chondrocytes, the second step in chondrogenesis (By similarity). Later, required to direct hypertrophic maturation and block osteoblast differentiation of growth plate chondrocytes: maintains chondrocyte columnar proliferation, delays prehypertrophy and then prevents osteoblastic differentiation of chondrocytes by lowering beta-catenin (CTNNB1) signaling and RUNX2 expression (By similarity). Also required for chondrocyte hypertrophy, both indirectly, by keeping the lineage fate of chondrocytes, and directly, by remaining present in upper hypertrophic cells and transactivating COL10A1 along with MEF2C (By similarity). Low lipid levels are the main nutritional determinant for chondrogenic commitment of skeletal progenitor cells: when lipids levels are low, FOXO (FOXO1 and FOXO3) transcription factors promote expression of SOX9, which induces chondrogenic commitment and suppresses fatty acid oxidation (By similarity). Mechanistically, helps, but is not required, to remove epigenetic signatures of transcriptional repression and deposit active promoter and enhancer marks at chondrocyte-specific genes (By

similarity). Acts in cooperation with the Hedgehog pathway-dependent GLI (GLI1 and GLI3) transcription factors (By similarity). In addition to cartilage development, also acts as a regulator of proliferation and differentiation in epithelial stem/progenitor cells: involved in the lung epithelium during branching morphogenesis, by balancing proliferation and differentiation and regulating the extracellular matrix (By similarity). Controls epithelial branching during kidney development (By similarity).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab220812 is the carrier-free version of ab185230.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility
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.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

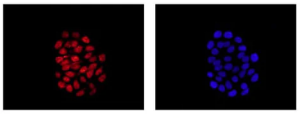
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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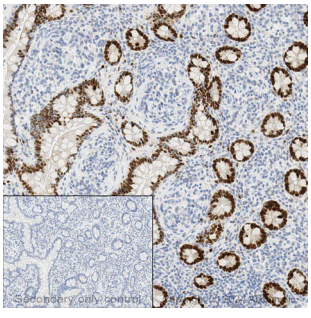
12 product images



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

SOX9 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-SOX9 antibody

Immunofluorescent analysis of SW480 cells (4% paraformaldehyd-fixed) labeling SOX9 with ab185230 at 1/250 dilution followed by Goat anti rabbit IgG (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 paraffin-embedded sections) - Anti-SOX9 antibody [EPR14335] - BSA and Azide free (ab220812)

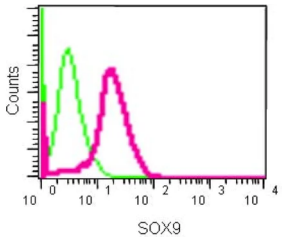
This data was developed using ab185230, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of formalin fixed paraffin embedded human colon labelling SOX9 with ab185230 at a concentration of 0.1µg/ml. The immunostaining was performed on a Ventana

DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with a OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was performed with DISCOVERY cell conditioning solution (CC1) 100°C, pH8.5 for 32mins.

Anti-SOX9 antibody [EPR14335] ab185230 was incubated for 16mins at 37°C. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

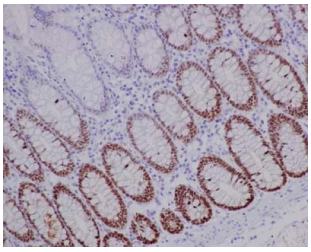
Customers are encouraged to optimise antigen retrieval conditions, antibody concentration, incubation times and temperature for best results in their own IHC assay workflow (automated and manual).



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

SOX9 Flow Cytometry (Intracellular) staining using rabbit Anti-SOX9 antibody

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

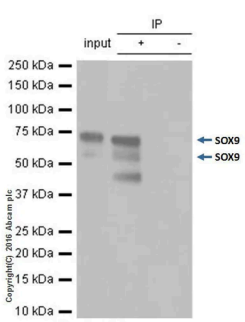


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SOX9 antibody [EPR14335] - BSA and Azide free (ab220812)

SOX9 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-SOX9 antibody

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 counter-stained 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)

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 IgG (ab172730) instead of ab185230 in SW480 whole cell lysate

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

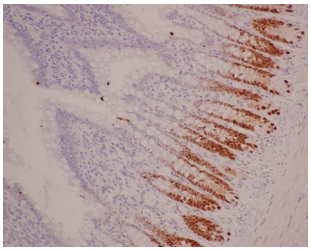
Blocking buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM /TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185230).
All lanes:
Immunoprecipitation - Anti-SOX9 antibody [EPR14335] (ab185230)

Predicted band size: 56 kDa

Observed band size: 56 kDa, 75 kDa

Exposure time: 10s

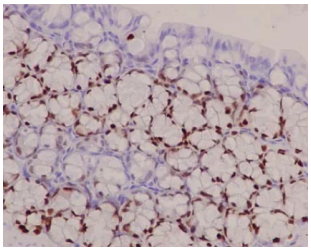


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SOX9 antibody [EPR14335] - BSA and Azide free (ab220812)

SOX9 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Rat colon tissue using rabbit Anti-SOX9 antibody

This data was developed using ab185230, the same antibody clone in a different buffer formulation.
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 counter-stained with Hematoxylin.

Heat mediated antigen retrieval was performed with EDTA buffer pH 9 before commencing with IHC staining protocol.



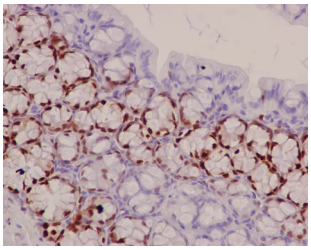
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SOX9 antibody [EPR14335] - BSA and Azide free (ab220812)

SOX9 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-SOX9 antibody

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 counter-stained 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.

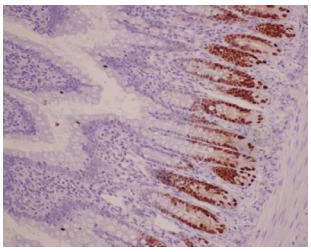


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SOX9 antibody [EPR14335] - BSA and Azide free (ab220812)

SOX9 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Mouse colon tissue using rabbit Anti-SOX9 antibody

This data was developed using ab185230, the same antibody clone in a different buffer formulation.
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 counter-stained with Hematoxylin.

Heat mediated antigen retrieval was performed with EDTA buffer pH 9 before commencing with IHC staining protocol.

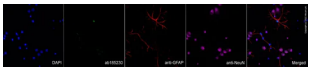


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SOX9 antibody [EPR14335] - BSA and Azide free (ab220812)

SOX9 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-SOX9 antibody

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

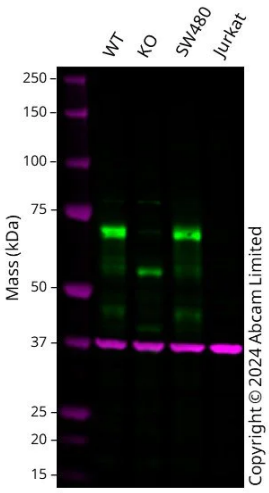
Perform heat mediated antigen retrieval with EDTA buffer pH 9 before commencing with IHC staining protocol.



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

SOX9 Immunocytochemistry/ Immunofluorescence staining of Primary hippocampal mouse neurons/glia DIV14. using rabbit Anti-SOX9 antibody

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 IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) ab150176 Goat Anti-Chicken IgY H&L (Alexa Fluor® 594) preadsorbed (shown in red) and ab150119 Goat Anti-Mouse IgG 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).



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

SOX9 Western blot staining using rabbit Anti-SOX9 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab185230).

Western blot: Anti-Sox9 antibody [EPR14335] (ab185230) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab185230 was shown to bind specifically to Sox9. A band was observed at 50-70 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in Sox9 CRISPR-Cas9 edited cell line. The band observed in the CRISPR-Cas9 edited lysate lane below 50-70 kDa is likely to represent a truncated form of Sox9. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and Sox9 CRISPR-Cas9 edited HCT 116 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

All lanes:
Western blot - Anti-SOX9 antibody [EPR14335] (ab185230) at 1/1000 dilution

Lane 1:
Wild-type HCT 116 cell lysate at 20 µg

Lane 2:
Sox9 CRISPR-Cas9 edited HCT 116 cell lysate at 20 µg

Lane 3:
SW480 cell lysate at 20 µg

Lane 4:

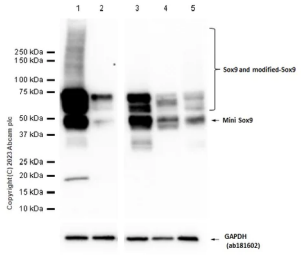
Jurkat cell lysate at 20 µg

Secondary

All lanes:
Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Observed band size: 50 kDa, 70 kDa



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

SOX9 Western blot staining using rabbit Anti-SOX9 antibody

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).

All lanes:
Western blot - Anti-SOX9 antibody [EPR14335] (ab185230) at 1/1000 dilution

Lane 1:
SW480 (Human colorectal adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 3:
NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate at 20 µg

Lane 4:
Mouse colon tissue lysate at 20 µg

Lane 5:
Mouse P0 bone tissue lysate at 20 µg

Secondary

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

Observed band size: 42 kDa, 56 kDa, 75 kDa

Exposure time: 40s

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.