abcam

ab76997

Anti-SOX9 antibody [3C10] - BSA and Azide free

Anti-SOX9 antibody [3C10] - BSA and Azide free (ab76997) is a mouse monoclonal antibody provided in a PBS only buffer for easy conjugation detecting SOX9 in **Western Blot, Flow Cytometry, IP, IHC-P, ICC/IF**. Suitable for **Human**.

- BSA, sodium azide, and glycerol-free for easy conjugation
- Over 50 publications
- Trusted since 2009

Key facts

Isotype	IgG2a
Host species	Mouse
Storage buffer	pH: 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	Recombinant Fragment Protein within Human Transcription factor SOX-9 aa 400 to C-terminus. The exact immunogen used to generate this antibody is proprietary information. Database link P48436
Carrier free	Yes
Clone number	3C10
Purification technique	Affinity purification Protein A
Light chain type	kappa
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	-
Notes	Perform heat-mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.

Not recommended

Species	Transfected cell lysate - Human
Dilution info	-
Notes	-

IP

Tested

Species	Transfected cell lysate - Human
Dilution info	-
Notes	-

Expected

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

Flow Cyt

Tested

Species	Human
Dilution info	-
Notes	ab170191 - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.

Not recommended

Species	Transfected cell lysate - Human
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Transfected cell lysate - Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Transfected cell lysate - Human
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	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

What is this antibody validated in?
Anti-SOX9 antibody [3C10] - BSA and Azide free (ab76997) is a mouse monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Flow Cyt), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF) in Human samples.

What is the molecular weight of SOX9?
Anti-SOX9 [3C10] - BSA and Azide free (ab76997) specifically detects a band for SOX9 (UniProt: P48436) at a molecular weight of 56kDa.

Trusted by the scientific community
Anti-SOX9 [3C10] - BSA and Azide free (ab76997) was first used in a scientific publication in 2009 and has been cited over 50 times in peer-reviewed journals.

Reviewed by scientists
Anti-SOX9 [3C10] - BSA and Azide free (ab76997) has over 15 independent reviews from customers.

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

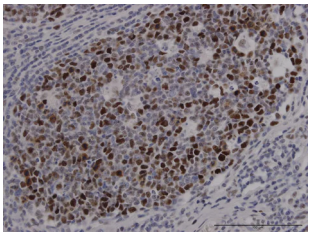
We do not recommend this combination. It is not covered by our product promise.

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.

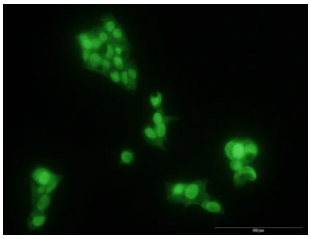
7 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SOX9 antibody [3C10] - BSA and Azide free (ab76997)

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

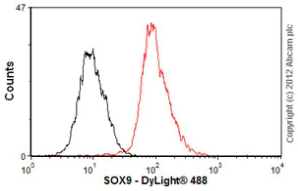
Immunoperoxidase analysis of SOX9 expression on formalin fixed, paraffin embedded human tonsil tissue using 0.7 µg/ml of ab76997.



Immunocytochemistry/ Immunofluorescence - Anti-SOX9 antibody [3C10] - BSA and Azide free (ab76997)

SOX9 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-SOX9 antibody

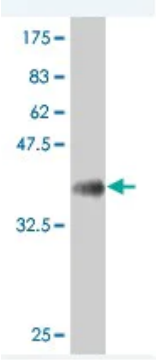
Immunocytochemistry/Immunofluorescence analysis of SOX9 expression in HepG2 cells, using 10 µg/ml of ab76997.



Flow Cytometry - Anti-SOX9 antibody [3C10] - BSA and Azide free (ab76997)

SOX9 Flow Cytometry staining using mouse Anti-SOX9 antibody

Overlay histogram showing HepG2 cells stained with ab76997 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab76997, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2a [ICIGG2A] (ab91361, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This image was generated using the ascites version of the product.



Western blot - Anti-SOX9 antibody [3C10] - BSA and Azide free (ab76997)

This image was generated using the ascites version of the product.
All lanes:
Western blot - Anti-SOX9 antibody [3C10] - BSA and Azide free (ab76997) at 5 µg/mL

All lanes:
immunogen at 0.2 µg

Secondary

All lanes:
Goat Anti-Mouse IgG (H&L)-HRP Conjugate at 1/5000 dilution

Predicted band size: 56 kDa

Observed band size: 38 kDa

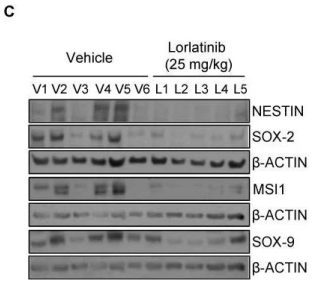


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Western blot - Anti-SOX9 antibody [3C10] - BSA and Azide free (ab76997)

SOX9 Western blot staining using mouse Anti-SOX9 antibody

Western Blotting using Anti-SOX9 antibody [3C10] - BSA and Azide free, ab76997. Publication image from López-Valero, I. et al., 2020, Theranostics, 32308772. Legend direct from paper.

Combination of MDK/ALK inhibitors with cannabinoids and TMZ strongly reduces the growth of GICs-derived xenografts. (A) Effect of daily oral administration of crizotinib (12.5 mg/kg, upper panel) or lorlatinib (25 mg/kg, bottom panel) on the volume of glioma xenografts generated by subcutaneous injection of 2 x 106 12O12-GICs (mean ± SEM on the last day of the treatment). **P < 0.01 from 12O12 vehicle-treated tumors. (B) Effect of daily oral administration of crizotinib (12.5 mg/kg) on the mRNA levels of NESTIN, MUSASHI-1 (MSI1), SOX2 and SOX9 (as determined by qPCR) of glioma xenografts generated by subcutaneous injection of 2 x 106 12O12-GICs (mean ± SEM). *P < 0.05 from 12O12 vehicle-treated tumors. (C) Effect of daily oral administration of lorlatinib (25 mg/kg) on the protein levels (as determined by Western blot) of NESTIN, MUSASHI-1 (MSI1), SOX2 and SOX9 in the tumor xenografts (V1-V6: vehicle-treated animals; L1-L5: lorlatinib-treated animals). (D) Effect of the treatment with crizotinib (0.5 μM) and TMZ (100 μM, upper panel or 20 μM bottom panel) on the self-renewal ability (as determined by LDA) of GH2 (upper panel) or 12O12 (bottom panel)-GICs (n=2). Full χ2 statistical analysis is included in LDA statistics section within the supplementary materials. (E) Effect of the treatment with THC:CBD (2.5 μM THC + 2.5 μM CBD) and MDK Ab. (40 μg/ml, left panel) or crizotinib (CZT, 0.25 μM, right panel) on the total number of cells (upper panels) and self-renewal capacity (bottom panels) of 12O12-GICs (n=3). **P < 0.01 and ***P < 0.001 from vehicle-treated cells; ###P < 0.001 from THC + CBD-treated cells; &P < 0.05 or &&P < 0.01 from MDK Ab.-treated cells or crizotinib-treated cells. Full χ2 statistical analysis is included in LDA statistics section within the supplementary materials. (F) Effect of the treatment with crizotinib (12.5 mg/kg daily oral administration) and TMZ (5 mg/kg twice a week IP administration) on the growth of glioma xenografts generated by subcutaneous injection of 2 x 106 12O12-GICs [mean ± SEM; n=5-6 mice for each condition]. Representative pictures of the tumor xenografts in the last day of the treatment are shown for each experimental condition. Symbols of significance are omitted for clarity except when the combined-treatment was significantly different from vehicle and each individual treatment (*P < 0.05 or **P <0.01 from each individual treatment). The rest of the statistical analysis can be found in Supplementary materials.

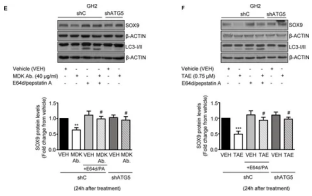


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Western blot - Anti-SOX9 antibody [3C10] - BSA and Azide free (ab76997)

SOX9 Western blot staining using mouse Anti-SOX9 antibody

Western Blotting using Anti-SOX9 antibody [3C10] - BSA and Azide free, ab76997. Publication image from López-Valero, I. et al., 2020, Theranostics, 32308772. Legend direct from paper.

Blockade of the MDK/ALK axis in GICs induces the autophagic degradation of SOX9. (A) LC3 and NESTIN immunostaining of GH2-GICs cells incubated with an anti-MDK antibody (MDK Ab., 40 μg/ml, 24 h) (n=3). Representative images (with a high magnification photomicrograph of the squared-pointed area of MDK Ab.-treated cells) are shown. Scale bar: 20 μm. (B) Quantification of the percentage of cells with LC3 dots within the population of NESTIN-positive or negative GH2-GICs (n=3). *P < 0.05 ***P < 0.001 from vehicle-treated cells; ##P < 0.01 from MDK Ab.-treated NESTIN-negative cells. ND: Non-detectable. (C) Effect of the incubation with an anti-MDK antibody (MDK Ab., 40 μg/ml) on MUSASHI1 (MSI1), CD133 and LC3-I/II protein levels of GH2-GICs stably transduced with a doxycycline-inducible shATG5 at different time points (n=3). A representative experiment is shown. (D) Effect of the incubation with an anti-MDK antibody (MDK Ab., 40 μg/ml) and the genetic inhibition of autophagy on the self-renewal ability (as determined by LDA) of GH2-GICs (n=2). #P < 0.05 from GH2 shATG5 (-Dox.) MDK Ab.-treated cells. Full χ2 statistical analysis is included in LDA statistics section within the supplementary materials. (E-F) Effect of the incubation with an anti-MDK antibody (MDK Ab., 40 μg/ml; 24 h, panel E) or TAE (0.75 μM, 24 h,

panel F) on SOX9 and LC3-I/II protein levels of shC or shATG5-transduced GH2-GICs cultures untreated or pretreated with E64d (10 µM) and pepstatin A (PA, 10 µg/ml) for 1 h. A representative Western blot (upper panels) and the corresponding densitometric quantifications (bottom panels) are shown (n=5). **P < 0.01 from vehicle-treated cells; #P < 0.05 from MDK Ab.-treated shC cells.

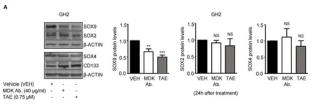


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MDK/ALK signaling axis regulates the maintenance of the stem-like properties of GICs by controlling SOX9 protein levels. (A) Effect of the incubation with an anti-MDK antibody (MDK Ab., 40 µg/ml) or TAE (0.75 µM) for 24 h on SOX2, SOX4, SOX9 and CD133 protein levels of GH2-GICs. Left panel: a representative Western blot experiment is shown (n=5). Right panel: densitometric analysis of SOX9, SOX2 and SOX4 protein levels (mean fold change from vehicle ± SEM; n=5 for SOX9 and SOX2 and n=3 for SOX4). **P < 0.01 and ***P < 0.001 from vehicle-treated cells. NS: statistically non-significant differences. (B) Effect of SOX9 genetic inhibition [by nucleofection with a plasmid encoding a shcontrol (shC) or a SOX9-selective (shSOX9) shRNA] on the self-renewal ability (as determined by LDA) of GH2-GICs (n=3) **P < 0.01 from shC cells. (C) Effect of SOX9 genetic inhibition (72 h) on mRNA levels of a panel of stem cell associated genes (as determined by qPCR) of GH2-GICs. Data are expressed as mean fold change from shC cells (reference) ± SEM (n=3). *P < 0.05; **P < 0.01; **P < 0.001 from shC cells. (D) Effect of the incubation with an anti-MDK antibody (MDK Ab., 40 µg/ml) and nucleofection with a control plasmid (CP), or a plasmid encoding a murine SOX9 (SOX9) on the self-renewal ability (as determined by LDA) of GH2-GICs (n=2). #P < 0.05 from MDK Ab.-treated CP cells. Full $\chi 2$ statistical analysis is included in LDA statistics section within the supplementary materials. (E) Effect of the incubation with an anti-MDK antibody (MDK Ab., 40 µg/ml) on the mRNA levels of NESTIN, MUSASHI-1 (MSI1) and SOX9 (as determined by qPCR) of GH2-GICs nucleofected with a control plasmid (CP) or a plasmid encoding a murine SOX9 (SOX9) (72 h). *P < 0.05 from vehicle CP-treated cells.

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