Bio'Ace BIOLOGICAL TECHNOLOGY
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Basic Information

Product Name	Anti-SOX9 Antibody (Clone# SN74-09)
Gene Name	SOX9
Source	Rabbit
Clonality	Monoclonal
Isotype	IgG
Species Reactivity	human, mouse, rat
Tested Application	WB, IF-Cell, IHC-P, IP, FC, CUT&Tag-seq, mIHC
Contents	1ug/ul in 1×TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Immunogen	Recombinant protein within human SOX9
Concentration	1ug/ul
Purification	Protein A affinity purified
Observed MW	Predicted band size: 56 kDa

Recommended Dilution Ratios

Application	Dilution Ratios
WB	1:10,000
IF-Tissue	1:1,000
IF-Cell	1:500
IHC-P	1:50-1:1,000
FC	1:2,000
IP	Use at an assay dependent concentration
mIHC	1:1,500

Storage

Store at +4°C after thawing. Aliquot store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.

Background Information

Transcription factor SOX-9 is a protein that in humans is encoded by the SOX9 gene. The protein encoded by this gene recognizes the sequence CCTTGAG along with other members of the HMG-box class DNA-binding proteins. It acts during chondrocyte differentiation and, with steroidogenic factor 1, regulates transcription of the anti-Muellerian hormone (AMH) gene. Deficiencies lead to the skeletal malformation syndrome campomelic dysplasia, frequently with sex reversal.

Selected Validation Data

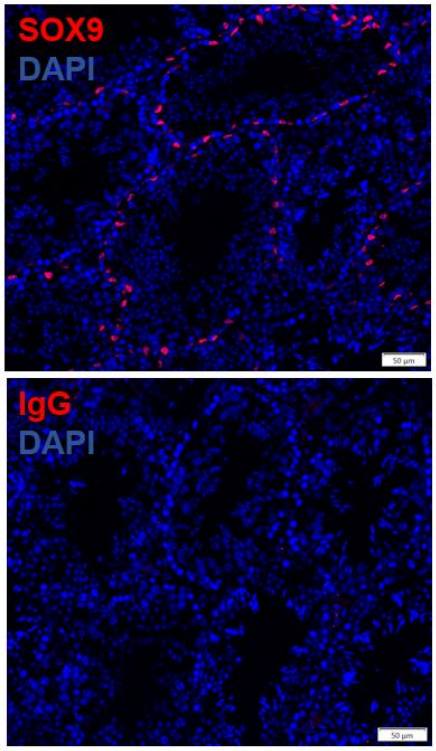

Figure 1. IF analysis of SOX9 using anti-SOX9 antibody (BA0001) in mouse testis. SOX9 was detected in a paraffin-embedded section of mouse testis tissue. 568 cy3 conjugated Anti- Rabbit IgG Secondary Antibody (Cyan) was used as secondary antibody. The section was counterstained with DAPI (Blue).

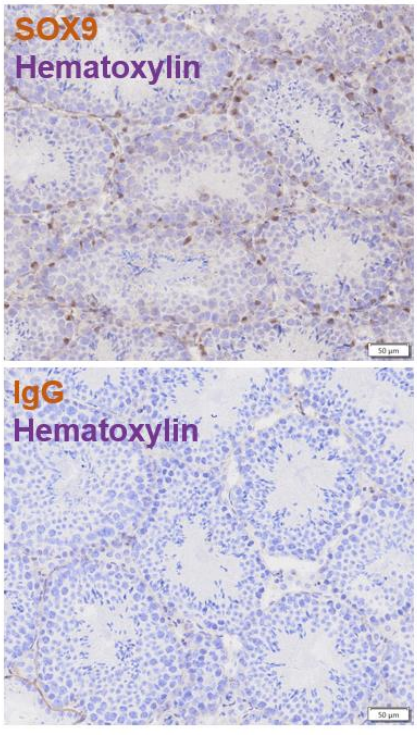

Figure 2. IHC analysis of SOX9 using anti-SOX9 antibody (BA0001) in mouse testis. SOX9 was detected in a paraffin-embedded section of mouse testis tissue. The tissue section was developed using HRP Conjugated Rabbit IgG with DAB as the chromogen.

Experiment Procedure

1. IF analysis of SOX9

(1) Reagent Preparation

Name	Batch Number/Source	Specifications
5%BSA	BAR0001	100g
10*PBS	BAR0002	500ml
TritonX-100	BAR0003	50ml
IGg	BAR0004/Rabbit	200μg
primary antibody	**BA0001/ Rabbit**	50μl
Secondary antibody	BAR0003 /Rabbit	0.5ml

Solution Preparation:

1x PBS: Dilute 200 ml of 10x PBS with ultrapure water to a total volume of 2 liters.

0.3% Triton X-100: Add 150 μl of Triton X-100 to 50 ml of PBS.

5% BSA: Dissolve 0.5 g of BSA in 10 ml of 1x PBS.

IgG Dilution Factor: For an antibody concentration of 1 mg/ml, the dilution factor is 1:200. For an IgG concentration of 1.67 mg/ml, the corresponding dilution factor is 1:334 (to maintain equal protein amounts).

(2) Experimental operation

1.Fixation: After harvesting the tissue (and rinsing thoroughly with PBS), immerse the tissue in FPA solution and store it at 4°C overnight. The next day, prepare 3% sucrose solution (15 g sucrose + 50 ml PBS, mix well) and add it to the centrifuge tube containing the tissue (just enough to cover the tissue, not too much). Store at 4°C overnight, replacing the 3% sucrose solution daily until the tissue sinks to the bottom of the tube. Then, mix 3% sucrose with OCT compound at a 1:1 ratio and store at 4°C overnight. The next day, retrieve the tissue for embedding.

2.Embedding: Take out the fixed tissue, use a blade to trim the tissue to the desired section, and place it in a PVC embedding mold with a layer of OCT compound (just enough to cover the bottom). Orient the tissue in the desired cutting direction (cutting surface facing down), then cover the tissue completely with OCT compound. Freeze in a -20°C freezer for 5 minutes.

3.Cryosectioning: Remove the cryostat's chuck and pre-cool the cryostat to -25°C. Demold the tissue block, attach it to the chuck using OCT compound, and freeze in a -50°C freezer for 5 minutes. Perform cryosectioning at a thickness of 5 μm. Use adhesive slides to pick up the sections (two spots per slide), label the slides, and place them on a staining rack. After sectioning, clean the cryostat (note: wipe the OCT compound off the chuck with paper).

4.Section selection: After cryosectioning, select slides with clear structures and good morphology (without folded, cracked, or incomplete sections). Place the slides in a 37°C oven for 10 minutes.

5.Gel washing: Pour 1x PBS into a staining jar and wash the slides in 1x PBS three times, each for 8 minutes. After

washing, gently blot off excess liquid with paper.

6.Membrane permeabilization: Place the slides in a humidified chamber, add 0.3% Triton, and incubate for 10 minutes. Then, wash the slides in 1x PBS on a shaker three times, each for 5 minutes. Membrane proteins do not require permeabilization (note: ensure the shaker speed is not too fast, as it may cause tissue detachment). After washing, gently blot off excess liquid with paper.

7.Blocking: Use a hydrophobic pen to circle the sample (draw a circle that is neither too large nor too small, avoiding the tissue itself to prevent damage). Place the slide in a humidified chamber, add 5% BSA, and incubate for 1 hour at room temperature. After incubation, gently blot off excess liquid with paper.

8.Primary antibody: Place the slide in a humidified chamber, add the primary antibody diluted in 5% BSA, and incubate overnight at 4°C (SOX9 dilution ratio 1:1000, i.e., 0.2 μl of primary antibody + 200 μl of 5% BSA, follow the manufacturer's recommendations for the dilution ratio). For control tissues, add IgG (IgG ratio: 0.1:167). Note: If there are two spots on one slide, select one spot as the control group. Use IgG that matches the species of the primary antibody. The next day, remove the slides from the 4°C refrigerator and wash them in 1x PBS on a shaker three times, each for 8 minutes. After washing, gently blot off excess liquid with paper.

9.Secondary antibody: Place the slide in a humidified chamber, add the secondary antibody diluted in 5% BSA, and incubate for 1 hour at room temperature, protected from light (SOX9 dilution ratio 1:400, i.e., 0.5 μl of secondary antibody + 200 μl of 5% BSA, channel: 568 Cy3). After incubation, wash the slide in 1x PBS on a shaker three times, each for 8 minutes, protected from light. After washing, gently blot off excess liquid with paper. (Note: Select a secondary antibody that is specific to the primary antibody. For example, if the primary antibody is from a rat, the secondary antibody should be anti-rat. Also, be mindful of the channel specified on the secondary antibody.)

10.Counterstaining: Add fluorescence mounting medium containing DAPI to the slide and counterstain for 10 minutes, protected from light (ensure the tissue is fully covered). Carefully check for air bubbles, and if present, gently remove them with a pipette without touching the tissue.

11.Mounting: Cover the tissue with a coverslip, and dry the slide in a 37°C oven for 30 minutes. When placing the coverslip, gently lower one side first, then slowly lower the other side by hand. Do this at a moderate speed to avoid creating air bubbles.

2. IHC analysis of SOX9

(1) Reagent Preparation

Name	Batch Number/Source	Specifications
5%BSA	BAR0001	100g
10*PBS	BAR0002	500ml
IGg	BAR0004/ Rabbit	200μg
primary antibody	**BA0001/ Rabbit**	50μl
DAB staining solution	BAR0006	18ml
TritonX-100	BAR0003	50ml
Tris-EDTA Antigen Retrieval Solution	BAR0007	100ml

Solution Preparation:

5% BSA: Dissolve 0.5 g of BSA in 7 ml of PBS, then dilute to a final volume of 10 ml.

1x PBS: Dilute 200 ml of 10x PBS with ultrapure water to a total volume of 2 liters, then add 500 μl of Tween.

IgG Dilution Factor: For an antibody concentration of 1 mg/ml, the dilution factor is 1:200. For an IgG concentration of 1.67 mg/ml, the corresponding dilution factor is 1:334 (to maintain equal protein amounts).

(2) Experimental operation

1.Tissue Processing: Immediately after removing the fresh tissue, fix it in 4% PFA at 4°C, protected from light, for 1–2 days.

2.Washing: Place the tissue into a disposable embedding cassette, label it with a pencil, and immerse it in a foam box filled with tap water for 30 minutes.

3.Waxing: Melt paraffin, paraffin-xylene mixture (1:1), Paraffin 1, and Paraffin 2 in a 65°C incubator. It usually takes a few hours to an entire afternoon. Check periodically; if smoke is observed, remove the paraffin and let it cool before continuing.

4.Gradient Dehydration: After soaking the tissue in tap water for 30 minutes, place it in 70% ethanol for 1 hour (for smaller organoids, adjust to 40 minutes). Use a P60 dish to prevent ethanol evaporation. The dehydration time depends on the size of the tissue block; larger blocks require longer times. Transfer the tissue to 80% ethanol for 1 hour, then to 95% ethanol for 40 minutes, and finally to 100% ethanol I/II, soaking for 1 hour each (twice).

5.Clearing: In a fume hood, immerse the tissue in a xylene-ethanol mixture (1:1) for 30 minutes (self-prepared). Use black section trays and operate inside embedding cassettes.

6.Xylene Immersion: Immerse the tissue in xylene in a fume hood for 20 minutes, repeating twice for a total of 40 minutes. After soaking, allow the tissue to air out in the fume hood for 20 minutes before removing.

7.Wax Impregnation: Soak the tissue in a paraffin-xylene mixture (1:1) at 65°C for 1 hour, then in Paraffin 1 at 65°C for 2 hours, and finally in Paraffin 2 at 65°C overnight for embedding.

8.Embedding: Check if there is enough wax in the embedding machine's reservoir, and add more if needed. Turn on the embedding machine, melt the wax on the metal embedding mold, then fill the mold with wax, carefully place the tissue inside, and adjust its orientation. Place the mold in the 4°C area, adjust the tissue orientation, and once the wax is semi-solidified, press a disposable plastic embedding cassette over the tissue. Cool for 3-4 minutes, then place the cassette in an ice box for an additional 3-4 minutes. Once fully solidified, remove the metal mold and immerse the plastic cassette (with the tissue) in ice water overnight. On 2024.8.8, add a bit more wax and ice for 1 hour.

9.Sectioning: (Preheat the 42°C water bath for spreading sections)

① Trim off excess wax from the tissue block and mount the block.

② Turn on the power and carefully mount the blade (left-side screw: adjust left-right positioning of the holder, align the blade with the wax block, use the blade from left to right; right-side screw: secure the blade).

③ Trim the block (20 μm thickness is typical for trimming; adjust with the +/- knob). Trim cautiously to avoid missing the target tissue. When trimming or sectioning, wet the block with water to facilitate cutting. Lock the handle after each cut for safety. Check the tissue periodically under a microscope to avoid cutting through the target. (After trimming, the block can be soaked in ice water for easier sectioning).

10.Sectioning and Spreading: Generally, cut 3–4 continuous sections per slide. Use the tip of the blade to break the connection between the top sections, then use tweezers to pick them up at a 45° angle and place them quickly onto a slide. Carefully pipette a small drop of 20% ethanol above the section (without touching the tissue), allow the tissue to naturally absorb the moisture, and then gently place the slide into the 42°C water bath. Once the sections are fully spread, immerse the slide face down into the water and retrieve the section, then label the slide (attach the label vertically). Observe under the microscope and decide whether to retain the section. After sectioning, remove and store the blade carefully, retrieve the next tissue, and clean the workspace. Turn off the machine, turn off the water bath, clean up debris, reset the holder, replace the microscope, and log usage. Place the collected slides in a 42°C or 37°C oven overnight.

11.Baking: Place slides with clear structures in a 65°C oven for 1 hour.

12.Deparaffinization: Immerse the slides in Deparaffinizing Solution 1 for 15 minutes, followed by Deparaffinizing Solution 2 for 15 minutes.

13.Rehydration: Immerse the slides in absolute ethanol for 5 minutes, 90% ethanol for 5 minutes, 70% ethanol for 5 minutes, and pure water for 5 minutes.

14.Antigen Retrieval: Use EDTA-TRIS/citric acid antigen retrieval solution, bring to a boil, and place the slides in the solution, reducing to low heat for 20 minutes. Allow to cool naturally to room temperature, and wash with PBS

for 5 minutes, twice.

15.Blocking: Incubate the slides with hydrogen peroxide at room temperature for 20 minutes, then wash with PBS for 5 minutes, twice.

16.Blocking (continued): Place the slides in a humidified chamber and add 5% BSA for 1 hour at room temperature. After incubation, gently blot off excess liquid with paper.

17.Primary Antibody: In the humidified chamber, add the primary antibody diluted in 5% BSA, and incubate overnight at 4°C (SOX9 dilution ratio 1:1000, i.e., 0.1 μl primary antibody + 100 μl 5% BSA; follow the antibody manual for reference). For control tissue, add IgG (IgG ratio: 1:1667). If the slide has three spots, select one as the control group. Use IgG matching the species of the primary antibody. The next day, remove the slides from the 4°C fridge and wash in 1x PBS on a shaker three times, each for 8 minutes. After washing, gently blot off excess liquid with paper.

18.Polymer: Place the slides in a humidified chamber and add enzyme-labeled anti-mouse/rabbit polymer. Incubate for 1 hour at room temperature. Afterward, wash with 1x PBS on a shaker three times for 5 minutes, protecting from light. After washing, blot off excess liquid with paper. (Note: the secondary antibody must match the primary. If the primary is from Rat, choose anti-Rat secondary antibody, and note the channel).

19.DAB Staining: Add freshly prepared DAB chromogen solution (1:20 or 1:40). Adjust staining time according to desired intensity, then immerse in ultrapure water for 5 minutes. (Time:30s)

20.Hematoxylin Counterstaining: Filter hematoxylin with a 0.45 mm filter before use. Adjust staining time based on desired intensity; if too dark, use a differentiation solution. Immerse in ultrapure water for 3 minutes. (Time:10s)

21.Dehydration: Place the slides in 95% ethanol for 5 minutes, then transfer to absolute ethanol for 5 minutes, repeating the absolute ethanol step.

22.Mounting: Place the slides in xylene until no bubbles remain. Add neutral resin and cover with a coverslip. Air dry for 15 minutes in the fume hood, then transfer to an oven for 2 hours (perform all steps in the fume hood).