

## Basic Information

|                    |   |
|--------------------|---|
| Product Name       | Anti-alpha smooth muscle Actin Antibody (Clone#SY02-64)                             |
| Gene Name          | alpha smooth muscle Actin   |
| Source             | Rabbit  |
| Clonality          | Monoclonal  |
| Isotype            | IgG   |
| Species Reactivity | human, mouse, rat   |
| Tested Application | WB, IF-Cell, IF-Tissue, IHC-P, FC, 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:42kDa   |

### Recommended Dilution Ratios

| Application | Dilution Ratios  |
|-------------|------------------|
| WB          | 1:5,000-1:50,000 |
| IF-Tissue   | 1:1,000          |
| IF-Cell     | 1:2,000          |
| IHC-P       | 1:50,000         |
| FC          | 1:1,000-1:2,000  |
| mIHC        | 1:2,000-1:10,000 |

## Storage

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

## Background Information

All eukaryotic cells express Actin, which often constitutes as much as 50% of total cellular protein. Actin filaments can form both stable and labile structures and are crucial components of microvilli and the contractile apparatus of muscle cells. While lower eukaryotes, such as yeast, have only one Actin gene, higher eukaryotes have several isoforms encoded by a family of genes. At least six types of Actin are present in mammalian tissues and fall into three classes.  $\alpha$ -Actin expression is limited to various types of muscle, whereas  $\beta$ -Actin and  $\gamma$ -Actin are the principle

constituents of filaments in other tissues. Members of the small GTPase family regulate the organization of the Actin cytoskeleton. Rho controls the assembly of Actin stress fibers and focal adhesion. Rac regulates Actin filament accumulation at the plasma membrane. Cdc42 stimulates formation of filopodia.

## Selected Validation Data

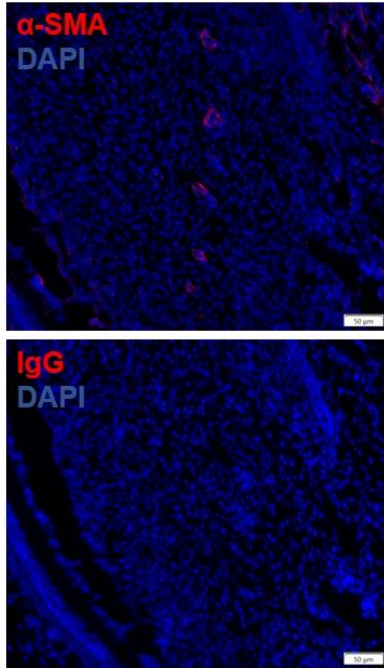


Figure 1. IF analysis of alpha smooth muscle Actin using anti-alpha smooth muscle Actin antibody (BA0003) in mouse teeth. Alpha smooth muscle Actin was detected in a paraffin-embedded section of mouse teeth tissue. 568 cy3 conjugated Anti- Rabbit IgG Secondary Antibody (Cyan) was used as secondary antibody. The section was counterstained with DAPI (Blue).

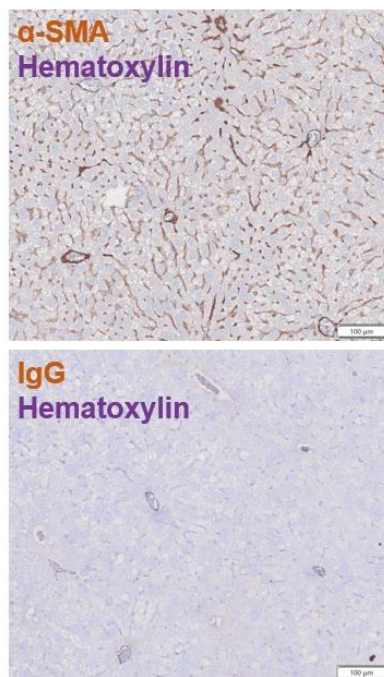


Figure 2. IHC analysis of alpha smooth muscle Actin using anti-alpha smooth muscle Actin antibody (BA0003) in mouse liver. Alpha smooth muscle Actin was detected in a paraffin-embedded section of mouse liver tissue. The tissue section was developed using HRP Conjugated Rabbit IgG with DAB as the chromogen.

# Experiment Procedure

## 1. IF analysis of alpha smooth muscle Actin

### (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   | <b>BA0003/ Rabbit</b> | 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 removing the tissue (and rinsing thoroughly with PBS), immerse the tissue in FPA solution and store it at 4°C overnight. The next day, prepare a 3% sucrose solution (15 g sucrose + 50 ml PBS, mix well), add it to a centrifuge tube containing the tissue (just enough to cover the tissue, not too much), and store it at 4°C overnight. Replace 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 it 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 desired section, and cut it to the required size. Add OCT compound to a PVC embedding mold (just enough to cover the bottom), place the tissue in the mold in the correct orientation for sectioning (cutting surface facing down), and fully cover the tissue with OCT compound. Freeze in a -20°C freezer for 5 minutes.

3. Cryosectioning: Remove the cryostat 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. Use the cryostat to section the tissue (slice thickness 10 µm), pick up the sections with adhesive slides (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. Slide Selection: After cryosectioning, select slides with clear structures and good morphology (without folds, cracks, 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 with 1x PBS three times, for 8 minutes each. After washing, gently blot off excess liquid with paper.
6. Permeabilization: Place the slides in a humidified chamber, add 0.3% Triton X-100, and incubate for 10 minutes. Then, wash the slides in 1x PBS on a shaker three times, for 5 minutes each. 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, ensuring it doesn't touch the tissue). 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 (alpha smooth muscle actin dilution ratio 1:400, i.e., 0.5 µl primary antibody + 200 µl 5% BSA; follow the antibody manual for reference). For control tissue, add IgG (IgG ratio: 0.5:334). Note: If the slide has two 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, for 8 minutes each. 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 (alpha smooth muscle actin dilution ratio 1:400, i.e., 1 µl secondary antibody + 400 µl 5% BSA; channel: 568 Cy3). After incubation, wash the slide in 1x PBS on a shaker three times, for 8 minutes each, protected from light. After washing, gently blot off excess liquid with paper. (Note: the secondary antibody must match the primary antibody. For example, if the primary is from Rat, select an anti-Rat secondary antibody and note the labeled channel 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; 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 alpha smooth muscle Actin

### (1) Reagent Preparation

| Name                                 | Batch Number/Source   | Specifications |
|--------------------------------------|-----------------------|----------------|
| 5%BSA                                | BAR0001               | 100g           |
| 10*PBS                               | BAR0002               | 500ml          |
| IGg                                  | BAR0004/ Rabbit       | 200µg          |
| primary antibody                     | <b>BA0003/ Rabbit</b> | 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 final 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 harvesting 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. This usually takes several hours to an entire afternoon. Check periodically, and if smoke is observed, remove the wax, wait for a while, then return it to the incubator.

4.Gradient Dehydration: After soaking the tissue in tap water for 30 minutes, place it in 70% ethanol for 1 hour (for smaller organoid tissues, adjust to 40 minutes). Use a P60 dish to prevent ethanol evaporation. Dehydration time depends on the size of the tissue block; larger blocks require longer dehydration. 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 self-prepared xylene-ethanol mixture (1:1) for 30 minutes. (Use the black section trays, and operate inside the 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, let the tissue air out in the fume hood for 20 minutes before removing it.

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: Ensure the embedding machine has sufficient wax. Turn on the embedding machine, melt the wax on the metal embedding mold, fill the mold with wax, carefully place the tissue inside, and adjust its orientation. Place the mold in the 4°C section, 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 (for as long as possible). On 2024.8.8, add a little more wax, and ice for 1 hour.

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

① Remove excess wax from the top and bottom of the tissue block, and mount the block.

② Turn on the power and carefully mount the blade. (Left screw: adjust the lateral positioning of the stage, align the blade with the paraffin block, and use the blade from left to right. Right screw: secure the blade.)

③ Trim the block to expose the tissue (trim thickness typically 20 µm, adjust with +/-). Trim cautiously to avoid missing the target tissue. When trimming or sectioning, wet the block with water to facilitate cutting. After each cut, lock the handle for safety. Observe under a microscope to avoid cutting through the target tissue. (After trimming, soaking the block in ice water helps with 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 place the sections quickly onto a slide at a 45° angle. Carefully pipette a small drop of 20% ethanol above the section (without touching the tissue), let the ethanol naturally absorb, then place the slide in the 42°C water bath. Once the sections are fully spread, retrieve the slide, label it vertically, and observe under the microscope. After cutting, remove and store the blade carefully, retrieve the next tissue, turn off the machine and water bath, clean the workspace, reset the stage, and log the 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. After bringing it to a boil, immerse the slides, reduce the heat for 20 minutes, and allow them to cool naturally to room temperature. Then wash with PBS for 5 minutes, twice.

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

16. Blocking: Place the slides in a humidified chamber, add 5% BSA, and incubate 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 (α-sma dilution ratio 1:500, i.e., 0.1 μl primary antibody + 100 μl 5% BSA; follow the antibody manual for guidance). For control tissue, add IgG (IgG ratio: 1:835). Note: 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, for 8 minutes each. After washing, gently blot off excess liquid with paper.
18. Polymer: In the humidified chamber, add enzyme-labeled anti-mouse/rabbit polymer, and incubate for 1 hour at room temperature. After incubation, wash the slides with 1x PBS on a shaker three times, for 5 minutes each, protected from light. After washing, gently blot off excess liquid with paper. (Note: the secondary antibody must match the primary antibody. For example, if the primary is from Rat, select an anti-Rat secondary antibody and ensure the correct channel is selected.)
19. DAB Staining: Add a sufficient amount of freshly prepared DAB chromogen solution (1:20 or 1:40). Adjust the staining time according to the desired intensity, then immerse in ultrapure water for 5 minutes. (Time:40s)
20. Hematoxylin Counterstaining: Filter hematoxylin through a 0.45 mm filter before use. Adjust staining time based on desired intensity (Time:10s); if too dark, use a differentiation solution. Immerse in ultrapure water for 3 minutes.
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, then add an appropriate amount of 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 inside the fume hood).