Anti- RUNX2 Antibody (Clone# SD208-0)

Catalog Number: *BA0009*

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Basic Information

Product Name	Anti-RUNX2 Antibody (Clone#SD208-0)
Gene Name	RUNX2
Source	Rabbit
Clonality	Monoclonal
Isotype	IgG
Species Reactivity	human, mouse, rat
Tested Application	IF-Cell, IF-Tissue, IHC-P, WB, FC, IP
Contents	1ug/ul in 1×TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Immunogen	Recombinant protein within human 300-450
Concentration	1ug/ul
Purification	Protein A affinity purified
Observed MW	57 kDa

Recommended Dilution Ratios

Application	Dilution Ratios
WB	1:5,000-1:10,000
IF-Tissue	1:50-1:200
IF-Cell	1:2,000-1:5,000
IHC-P	1:200-1:1,000
FC	1:5,000
IP	1-2µg/sample

Storage

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

Background Information

The mammalian Runt-related transcription factor (RUNX) family comprises three members, RUNX1 (also designated AML-1, PEBP2αB, CBFA2), RUNX2 (also designated AML-3, PEBP2αA, CBFA1, Osf2) and RUNX3 (also designated AML-2, PEBPαC, CBFA3). RUNX family members are DNA-binding proteins that regulate the expression of genes involved in cellular differentiation and cell cycle progression. RUNX2 is essential for skeletal mineralization in that it stimulates osteoblast differentiation of mesenchymal stem cells, promotes chondrocyte hypertrophy and contributes to endothelial cell migration and vascular invasion of developing bones. Regulating RUNX2 expression may be a

useful therapeutic tool for promoting bone formation. Mutations in the C-terminus of RUNX2 are associated with cleidocranial dysplasia syndrome, an autosomal-dominant skeletal dysplasia syndrome that is characterized by widely patent calvarial sutures, clavicular hypoplasia, supernumerary teeth, and short stature.

Selected Validation Data

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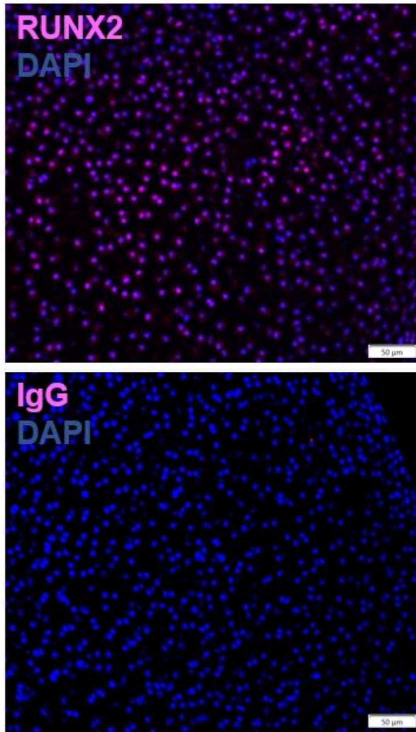
<td>
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<td>Figure 1. IF analysis of RUNX2 using anti- RUNX2 antibody (BA0009) in mouse bone.RUNX2 was detected in a paraffin-embedded section of mouse bone tissue. 568 cy3 conjugated Anti- Rabbit IgG Secondary Antibody (Cyan) was used as secondary antibody. The section was counterstained with DAPI (Blue).</td>
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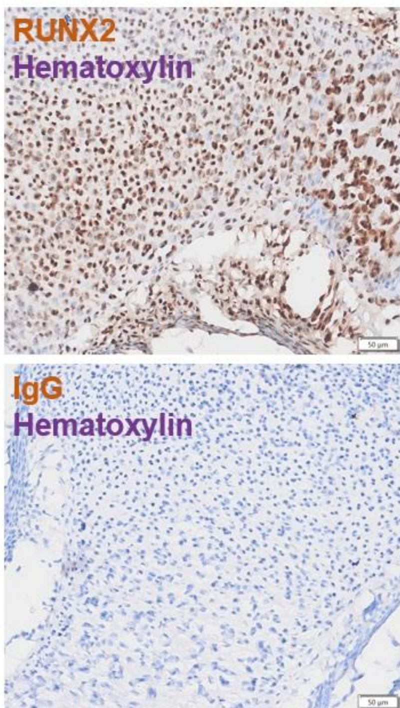
<td>
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<td>Figure 2. IHC analysis of RUNX2 using anti- RUNX2 antibody (BA0009) in mouse bone. RUNX2 was detected in a paraffin-embedded section of mouse bone tissue. The tissue section was developed using HRP Conjugated Rabbit IgG with DAB as the chromogen.</td>
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Experiment Procedure

1. IF analysis of RUNX2

(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	**BA0009/ 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 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, store it at 4°C overnight, and retrieve the tissue the next day 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 it 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 (RUNX2 dilution ratio 1:200, i.e., 0.5 μl primary antibody + 100 μl 5% BSA; follow the antibody manual for reference). For control tissue, add IgG (IgG ratio: 0.5:167). 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 (RUNX2 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: Add fluorescence mounting medium, 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 RUNX2

(1) Reagent Preparation

Name	Batch Number/Source	Specifications
5%BSA	BAR0001	100g
10*PBS	BAR0002	500ml
IGg	BAR0004/ Rabbit	200μg
primary antibody	**BA0009/ 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: After harvesting the fresh tissue, immediately fix it in 4% PFA at 4°C, protected from light, for 1-2 days.

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

3.Wax Preparation: Melt paraffin, the xylene-paraffin mixture (1:1), Paraffin 1, and Paraffin 2 in a 65°C incubator. This process usually takes several hours to an entire afternoon. Check periodically, and if smoke appears, remove the wax and wait for a while before returning it to the incubator.

4.Gradient Dehydration: After soaking the tissue in tap water for 30 minutes, transfer it to 70% ethanol for 1 hour (for small organoid tissues, adjust to 40 minutes). Use a P60 dish to prevent ethanol evaporation. Dehydration time varies depending on the size of the tissue block; larger blocks may require extended dehydration times. Then immerse the tissue in 80% ethanol for 1 hour, 95% ethanol for 40 minutes, and 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 black trays and embedding cassettes for operation).

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 dry in the fume hood for 20 minutes before removing it.

7.Wax Embedding: Immerse the tissue in a xylene-paraffin mixture (1:1) at 65°C for 1 hour, then Paraffin 1 at 65°C

for 2 hours, followed by Paraffin 2 at 65°C overnight for embedding.

8.Embedding: Check the wax level in the embedding machine and add more wax if necessary. Turn on the embedding machine, melt the wax on the metal embedding mold, fill the mold with wax, and carefully place the tissue inside, adjusting its orientation. Transfer the mold to the 4°C section and adjust the tissue orientation. Once the wax is semi-solid, 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 August 8, 2024, add a bit more wax and ice for 1 hour.

9.Sectioning (Preheat the 42°C Water Bath for Stretching Sections):

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

②Turn on the power and carefully mount the blade. (Left screw: adjust the lateral position of the stage and align the blade with the paraffin block. Right screw: secure the blade in place.)

③Trim the block to expose the tissue (trim thickness is usually 20 μm, adjust using +/-). Trim cautiously to avoid missing the target tissue. Wet the block with water to aid sectioning. After each cut, lock the handle for safety. Observe under a microscope to avoid missing the target tissue. (After trimming, soaking the block in ice water can facilitate sectioning.)

10.Sectioning and Stretching: Generally, cut 3-4 continuous sections per slide. Use the tip of the blade to break the connection between the 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 stretched, retrieve the slide, label it vertically, and observe under a microscope. After cutting, carefully remove and store the blade, 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, and incubate for 20 minutes. Allow the slides 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: Place the slides in a humidified chamber, add the primary antibody diluted in 5% BSA, and incubate overnight at 4°C (Runx2 dilution ratio 1:200, i.e., 0.1 μl primary antibody + 100 μl 5% BSA; follow the antibody manual for reference). For control tissue, add IgG (IgG ratio: 1:334). 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: Place the slides in a 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 the slides in ultrapure water for 5 minutes. (Time:25s)

20.Hematoxylin Counterstaining: Filter hematoxylin through a 0.45 mm filter before use. Adjust staining time based on desired intensity; if the color is too dark, use a differentiation solution. Immerse the slides 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, 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).