

Basic Information

Product Name	Anti-S100beta Antibody (Clone#SN74-09)
Gene Name	S100beta
Source	Rabbit
Clonality	Monoclonal
Isotype	IgG
Species Reactivity	Human, Mouse, Zebrafish, Rat, Goat
Tested Application	WB, IF-Cell, IF-Tissue, IP, IHC-P, IHC-Fr
Contents	1ug/ul in 1xTBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Immunogen	Synthetic peptide within C-terminal human S100 beta.
Concentration	1ug/ul
Purification	Protein A affinity purified
Observed MW	11 kDa

Recommended Dilution Ratios

Application	Dilution Ratios
WB	1:1,000-1:2,000
IF-Tissue	1:50-1:200
IF-Cell	1:50-1:200
IHC-P	1:50-1:1,000
IP	Use at an assay dependent concentration
IHC-Fr	1:100

Storage

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

Background Information

The protein encoded by this gene is a member of the S100 family of proteins containing 2 EF-hand calcium-binding motifs. S100 proteins are localized in the cytoplasm and/or nucleus of a wide range of cells, and involved in the regulation of a number of cellular processes such as cell cycle progression and differentiation. S100 genes include at least 13 members which are located as a cluster on chromosome 1q21; however, this gene is located at 21q22.3. This protein may function in Neurite extension, proliferation of melanoma cells, stimulation of Ca²⁺ fluxes, inhibition of PKC-mediated phosphorylation, astrocytosis and axonal proliferation, and inhibition of microtubule assembly. Chromosomal rearrangements and altered expression of this gene have been implicated in several neurological,

neoplastic, and other types of diseases, including Alzheimer's disease, Down's syndrome, epilepsy, amyotrophic lateral sclerosis, melanoma, and type I diabetes.

Selected Validation Data

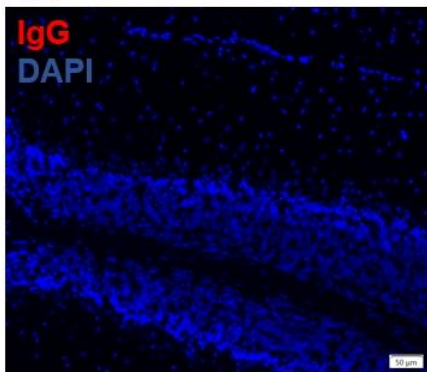
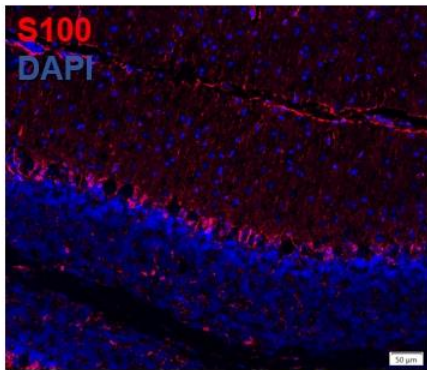


Figure 1. IF analysis of S100beta using anti- S100beta antibody (BA0010) in mouse cerebellum. S100beta was detected in a paraffin-embedded section of mouse cerebellum tissue. 568 cy3 conjugated Anti- Rabbit IgG Secondary Antibody (Cyan) was used as secondary antibody. The section was counterstained with DAPI (Blue).

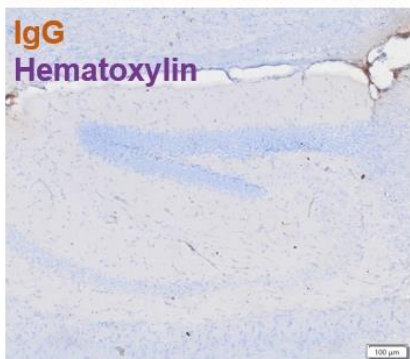
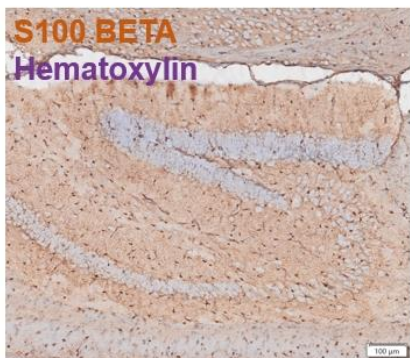


Figure 2. IHC analysis of S100beta using anti-S100beta antibody (BA0010) in mouse hippocampus. S100beta was detected in a paraffin-embedded section of mouse hippocampus tissue. The tissue section was developed using HRP Conjugated Rabbit IgG with DAB as the chromogen.

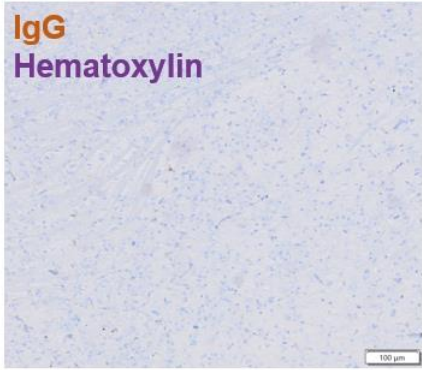
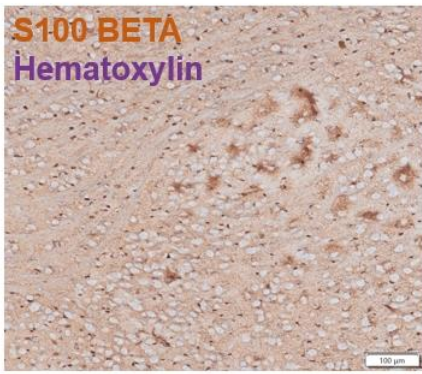


Figure 3. IHC analysis of S100beta using anti-S100beta antibody (BA0010) in mouse brain. S100beta was detected in a paraffin-embedded section of mouse brain tissue. The tissue section was developed using HRP Conjugated Rabbit IgG with DAB as the chromogen.

Experiment Procedure

1. IF analysis of S100beta

(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	BA0010/ 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 (S100beta dilution ratio 1:100, i.e., 1 µl primary antibody + 100 µl 5% BSA; follow the antibody manual for reference). For control tissue, add IgG (IgG ratio: 1:167). 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 (S100beta 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 S100beta

(1) Reagent Preparation

Name	Batch Number/Source	Specifications
5%BSA	BAR0001	100g
10*PBS	BAR0002	500ml
IGg	BAR0004/ Rabbit	200µg
primary antibody	BA0010/ 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 removing 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, a 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 before placing it back in 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 depends on the size of the tissue block; larger blocks may require longer dehydration. Immerse the tissue in 80% ethanol for 1 hour, 95% ethanol for 40 minutes, and 100% ethanol I/II for 1 hour 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, let the tissue 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, and finally 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 (containing the tissue) in ice water overnight (preferably as long as possible). On August 8, 2024, a small amount of additional wax was applied and iced 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 (S100beta 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). 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:20s)

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).