Bio'Ace BIOLOGICAL TECHNOLOGY
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Basic Information

Product Name	Anti-DCAMKL1 Antibody (Clone#JA11-03)
Gene Name	DCAMKL1
Source	Rabbit
Clonality	Monoclonal
Isotype	IgG
Species Reactivity	human, mouse, rat
Tested Application	WB, IHC-P, IF-Cell, FC
Contents	1ug/ul in 1×TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Immunogen	Synthetic peptide within Human DCAMKL1
Concentration	1ug/ul
Purification	Protein A affinity purified
Observed MW	Predicted band size: 82 kDa

Recommended Dilution Ratios

Application	Dilution Ratios
WB	1:1,000-1:2,000
IF-Tissue	1:200
IF-Cell	1:100
IHC-P	1:1,000
FC	1:1,000

Storage

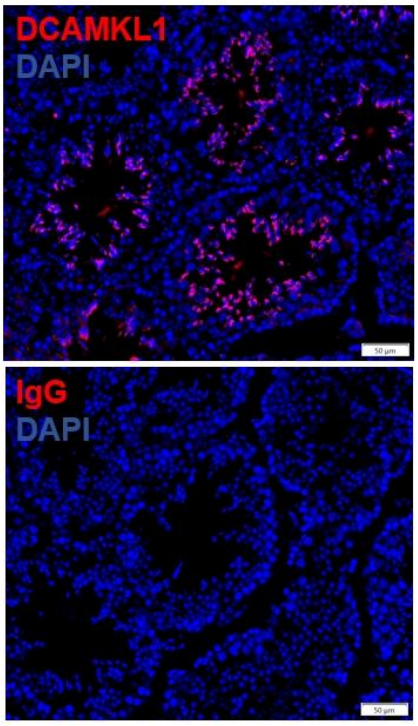
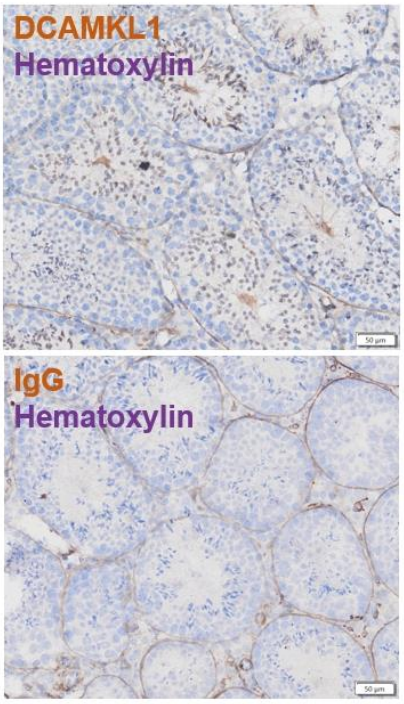
Store at +4°C after thawing. Aliquot store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.

Background Information

Lissencephaly (smooth brain) is an abnormality of brain development characterized by incomplete neuronal migration and a smooth cerebral surface, manifesting as severe mental retardation. Genetic analysis has identified two proteins that are mutated in some cases of lissencephaly, designated lissencephaly-1 protein (LIS1) and doublecortin. LIS1 displays sequence homology to β-subunits of heterotrimeric G proteins, and doublecortin contains a consensus Abl phosphorylation site. In addition, the DCAMKL1 (doublecortinlike and CAM kinase-like 1) protein shows homology to doublecortin. All three proteins are highly expressed in developing brain and may function together to regulate microtubules involved in neuronal migration. The DCAMKL1 protein encodes a functional kinase that is capable of phosphorylating myelin basic protein and itself, but its kinase activity does not appear to affect

its microtubule polymerization activity.

Selected Validation Data

	Figure 1. IF analysis of DCAMKL1 using anti- DCAMKL1 antibody (BA0006) in mouse testis. DCAMKL1 was detected in a paraffin-embedded section of mouse testis tissue.647 cy5 conjugated Anti- Rabbit IgG Secondary Antibody (Cyan) was used as secondary antibody. The section was counterstained with DAPI (Blue).
	Figure 2. IHC analysis of DCAMKL1 using anti-DCAMKL1 antibody (BA0006) in mouse testis. DCAMKL1 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 DCAMKL1

(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	**BA0006/ 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 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 5 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 (DCAMKL1 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 (DCAMKL1 dilution ratio 1:400, i.e., 1 μl secondary antibody + 400 μl 5% BSA; channel: 647 Cy5). 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 DCAMKL1

(1) Reagent Preparation

Name	Batch Number/Source	Specifications
5%BSA	BAR0001	100g
10*PBS	BAR0002	500ml
IGg	BAR0004/ Rabbit	200μg
primary antibody	**BA0006/ 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 harvesting the fresh tissue, 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.Waxing: Melt paraffin, paraffin-xylene mixture (1:1), Paraffin 1, and Paraffin 2 in a 65°C incubator. This process typically takes several hours or 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 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 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 there is sufficient wax in the embedding machine's reservoir; add more wax if needed. 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. 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 bit 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.

②Turn 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.)

③Trim 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 (Dcamkl1 dilution ratio 1:200, i.e., 0.1 μl primary antibody + 100 μl 5% BSA; follow the antibody manual for guidance). For control tissue, add IgG (IgG ratio: 1:334). 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: 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:30s)

20.Hematoxylin Counterstaining: Filter hematoxylin through 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, 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 in the fume hood).