Rat Brain Microvascular Endothelial Cell Isolation and Culture Kit

Cat. No.: P-CA-601

Size: 3Tests/10Tests

Background

The Rat Brain Microvascular Endothelial Cell Isolation and Culture Kit is specifically developed for the extraction of primary rat brain microvascular endothelial cells. As validated, standard operation using this kit enables the acquisition of one flask of cells (T-25 culture flask) per 1 Test, with a cell count exceeding 1×10^6 cells. When passaged at a 1:2 ratio, the cells can undergo 1-2 passages. Through immunofluorescence analysis, the cell purity (CD31-positive rate) has been confirmed to exceed 90%.

Scope of Application

This product is suitable for isolating brain microvascular endothelial cells from 14-day-old rats of various strains, such as Wistar and SD. Through processes of tissue isolation, enzymatic digestion, and 48-hour planting purification, a yield of $>1\times10^6$ cells can be obtained.

Note: The extraction of intact brain tissue from 3 rats (6 intact cerebral hemispheres) is required to yield sufficient cells for one T-25 flask. The exact number of rats required may vary depending on the size and quantity of brain tissue harvested during the procedure.

Kit Components

Name	Size	Appearance	Storage and Expiration Date
Specialized Washing Solution For Rat Brain Microvascular Endothelial Cells	3Tests (250 mL) 10Test (500 mL×2)	Faint Yellow Clear Liquid	2-8℃, 1 year
Specialized Digestive Solution A For Rat Brain Microvascular Endothelial Cells	3Tests (15 mL) 10Tests (50 mL)	Yellow Clear Liquid	-5~-20℃, 1 year
Specialized Digestive Solution B For Rat Brain Microvascular Endothelial Cells	3Tests (1.8 mL) 10Tests (6 mL)	Colorless Clear Liquid	-5~-20℃, 1 year
Specialized Isolation Solution For Rat Brain Microvascular Endothelial Cells	3Tests (15 mL) 10Tests (50 mL)	Yellow Clear Liquid	2-8℃, 1 year
Basic Culture Medium For Rat Brain Microvascular Endothelial Cells	3Tests (50 mL) 10Tests (100 mL)	Red Clear Liquid	2-8℃, 1 year
Supplement For Rat Brain Microvascular Endothelial Cells	3Tests (5 mL) 10Tests (10 mL)	Yellow Clear Liquid	-5~-20℃, 1 year
Planting Solution For Rat Brain Microvascular Endothelial Cells	3Tests (3 mL) 10Tests (10 mL)	Colorless Clear Liquid	-5~-20℃, 1 year
Screening Solution For Rat Brain Microvascular Endothelial Cells	3Tests (60 μL) 10Tests (200 μL)	Colorless Clear Liquid	-5~-20℃, 1 year
100 μm Cell Filter	3Tests (3 pcs) 10Tests (10 pcs)	Green	Room temperature, 3 years

Note: All components should be stored according to the temperature indicated on the labels of the reagent tubes. The reagents stored at -5~-20℃ (such as Specialized Digestive Solution for Rat Brain Microvascular Endothelial Cells) can be preserved at 4℃ for 30 days after thawing. For long-term storage, aliquot them into single-use portions and freeze at -5~-20℃. Thaw again before use to avoid repeated freeze-thaw cycles.

Note

1. Prior to formal experiments, it is recommended to conduct anatomical simulation training using 1-2 normal rats to familiarize operators with procedural workflows and improve tissue dissociation efficiency.
2. During the entire tissue dissociation process, place the small dish containing the tissue on an ice tray/ice box (2-8℃) to maintain hypothermic conditions. Critical precautions: Monitor temperature rigorously to prevent ice crystal formation in tissues/liquids.
3. Reagent preparation or dispensing must strictly adhere to aseptic technique protocols. After dispensing, seal the containers immediately with a sealing film, use them promptly to avoid repeated freeze-thaw cycles or contamination.

Operational Procedures

1. Pre-experiment Preparation

1) Self-supplied Reagents and Consumables: two Eppendorf (EP) tube racks; one ice tray/ice plate; Phosphate-Buffered Saline (PBS); surgical instruments (At least 3 pairs of ophthalmic scissors; 1 pair of straight forceps; 2 pairs of curved forceps; 1 pair of micro straight forceps; 1 pair of micro curved forceps); 6 cm/10 cm culture dishes; T25 culture flask; dissection board (foam board substitute); assorted 2 mL/15 mL/50 mL centrifuge tubes.
2) Reagent Thawing and Rewarming:
   ① Specialized Digestive Solution A For Rat Brain Microvascular Endothelial Cells; Specialized Digestive Solution B For Rat Brain Microvascular Endothelial Cells; Supplement For Rat Brain Microvascular Endothelial Cells; Planting Solution For Rat Brain Microvascular Endothelial Cells; Screening Solution For Rat Brain Microvascular Endothelial Cells: Thaw at 4℃ and equilibrate to room temperature.
   ② Specialized Washing Solution For Rat Brain Microvascular Endothelial Cells; Specialized Isolation Solution For Rat Brain Microvascular Endothelial Cells; Basic Culture Medium For Rat Brain Microvascular Endothelial Cells: Equilibrate to room temperature.
3) Preparation of complete culture medium: Add 5 mL of Supplement For Rat Brain Microvascular Endothelial Cells into 50 mL of Basic Culture Medium For Rat Brain Microvascular Endothelial Cells, mix thoroughly.
   Note: Complete culture medium: 2–8℃, valid for 3 months. When preparing complete culture medium, it can be prepared according to the usage amount. Remaining additives should be aliquoted proportionally and stored at -5~-20℃ to avoid repeated freeze-thaw cycles.
4) Coating of Culture Vessels: Add 1 mL of Rat Brain Microvascular Endothelial Cell Planting Solution into a T25 culture flask. Gently swirl to ensure even coverage of the bottom surface. Incubate the flask in a 37℃, 5% CO2 incubator for 0.5–2 hours.

2. Dissection Protocol

1) Animal Sterilization and Euthanasia: Perform euthanasia via pentobarbital sodium overdose injection or cervical dislocation, then immerse the animal in 75% medical-grade ethanol for 5 minutes for disinfection. After sterilization, transfer the animal to a clean bench for subsequent procedures.
2) Dissection and Tissue Harvesting Steps:
   ① Preparation: Arrange sterilized scissors and forceps in pairs (ophthalmic scissors and straight/curved forceps) from left to right on two sterilized EP tube racks: Ophthalmic Scissors 1 and Straight Forceps 1; Ophthalmic Scissors 2 and Curved Forceps 2; Ophthalmic Scissors 3 and Curved Forceps 3.
   Note: The distal third of the instruments should extend beyond the rack to avoid contamination. After each use, return tools to their original positions and make sure they don't touch each other to prevent cross-contact.
   ② Rat fixation: Secure the rat in a prone position within the clean bench using needles for stabilization

during tissue harvesting.

③ Tissue Sampling:

a. Using Straight Forceps 1 to grasp the dorsal skin, cut along the midline from the back to the nasal bridge using Ophthalmic Scissors 1, extending downward to the mandible. Reflect the skin laterally to fully expose the skull.

Note: When the skin is cut to expose the eyeball, avoid hair contamination by tearing the fur away from the dissection area.

b. Use Straight Forceps 1 to clamp the mouth of the rat vertically and fix it, use Ophthalmic Scissors 2 to cut the cervical vertebrae from the neck, and use Ophthalmic Scissors 2 to cut the skull along the cervical vertebrae incision toward the middle of the skull.

Note: Do not insert the scissors too deep into the inside of the head. Cut lightly upward and forward to avoid cutting the brain tissue under the skull.

c. Secure the mouth vertically with Straight Forceps 1. Use Ophthalmic Scissors 2 to sever the connection between the skull and the skull base on both sides. Cut the olfactory bulbs along the midline black line of the orbit, then carefully peel the skull open with Curved Forceps 2.

Note: Use Curved Forceps 2 to clamp only the skull, and avoid clamping the brain tissue to prevent it from being crushed or contaminated.

d. Gently lift the brain tissue with Curved Forceps 3 and transfer it to a culture dish containing 10 mL of Specialized Washing Solution For Rat Brain Microvascular Endothelial Cells (Figure 1). Place the dish on an ice tray/ice box to maintain a low-temperature environment.

Note: Only the first set of instruments may contact external skin; Other instruments are strictly prohibited from touching external skin and hair. If they do, sterile instruments must be replaced to prevent contamination. Periodically agitate the dish to prevent tissue freezing during prolonged procedures.

3. Tissue Processing and Digestion

1) Tissue Processing

① Put straight micro forceps and curved micro forceps on the EP tube rack within the biosafety cabinet, ensuring their tips remain suspended.

② Brain tissue dissection: Tissue dissection with the new micro forceps set, fixate the brain tissue with straight micro forceps in the left hand. Separate the olfactory bulb (anterior brain) using curved micro forceps in the right hand (Figure 2). Isolate the cerebrum from the cerebellum (Figure 3). Bisect the cerebrum along the midline to separate left and right hemispheres (Figure 4).

③ Cortical tissue purification: Flip the hemispheres (Figure 5) and remove the medulla, retaining only the cerebral cortex (Figure 6). Gently scrape visible vascular structures from the cortical surface using micro forceps to obtain purified cortical tissue (Figure 7).

④ Tissue fragmentation: Transfer the processed cortical tissue into three 2 mL microcentrifuge tubes. Add 0.5 mL Specialized Washing Solution For Rat Brain Microvascular Endothelial Cells to each tube. Rapidly mince the tissue into about 1 mm^3 fragments using Ophthalmic Scissors 3 (about 200 cuts required). Transfer the fragments to a 15 mL centrifuge tube using a 5 mL pipette or Pasteur Pipette. Add 10 mL Specialized Washing Solution For Rat Brain Microvascular Endothelial Cells to resuspend tissue fragments. Then centrifuge at 1200 rpm for 1 min (room temperature). Discard supernatant, and retain pellet.

2) Tissue Digestion

① Primary digestion: Add 5 mL Specialized Digestive Solution A For Rat Brain Microvascular Endothelial

Cells to the pellet. Mix gently and transfer the suspension to a 6 cm Petri dish. Incubate at 37℃, 5% CO_2 for 40 min.

② Secondary digestion: Add 0.6 mL Specialized Digestive Solution B For Rat Brain Microvascular Endothelial Cells to the dish. Pipette up and down gently about 10 times using a 5 mL pipette or Pasteur Pipette. Return to the incubator for another 40 min.

③ Termination of digestion: After incubation, pipette the suspension gently for 30 times. Add 5 mL Washing Solution to neutralize digestion enzymes.

3) Cell Isolation

① Place a 100 μm Cell Filter on a new 50 mL centrifuge tube. Pre-wash the filter with 3–5 mL washing solution.

② Slowly load the digested suspension onto the filter using a pipette. Collect filtrate in the 50 mL tube. Rinse the filter with 3–5 mL washing solution to maximize cell recovery.

Note: If filtration is impeded, slightly tilt the filter to reduce vacuum sealing against the tube rim.

③ Centrifugation and separation: Centrifuge the filtrate at 800× g for 5 min. Discard the supernatant and keep the precipitate. Add 5 mL Specialized Isolation Solution For Rat Brain Microvascular Endothelial Cells to resuspend the pellet. Transfer to a 15 mL tube and centrifuge at 1000× g for 30 min.

④ Layer separation: Post-centrifugation, three distinct layers will form: Upper layer: tissue debris. Middle layer: isolation solution. Lower layer: cell pellet. Use a 5 mL pipette or Pasteur pipette to carefully remove all the upper tissue layers, then replace a clean pipette tip and remove the middle layer of separation solution as completely as possible, leaving the precipitate below.

⑤ Final cell preparation: Resuspend the pellet in 0.5 mL washing solution and transfer to a new 15 mL tube. Add 5 mL washing solution and centrifuge at 1200 rpm for 5 min. Discard supernatant, retain the purified endothelial cell pellet.

4. Cell Culture and Subculture

1) Cell seeding: Take out the pre-coated T25 cell culture flask and aspirate the Planting Solution For Rat Brain Microvascular Endothelial Cell. Slowly add 5 mL of Specialized Washing Solution For Rat Brain Microvascular Endothelial Cells along the inner wall of the flask to avoid disrupting the coated surface. Aspirate the washing solution after gentle rinsing. Resuspend the cell pellet in 5 mL of Complete Medium For Rat Brain Microvascular Endothelial Cells and transfer the suspension into the coated T25 flask. Add 20 μL of Rat Brain Microvascular Endothelial Cell Screening Working Solution. Incubate at 37℃ with 5% CO2 for static culture.

Note: Initial seeding yields approximately 2.5×10^6 cells, with $>1\times10^6$ viable cells after 48 h purification.

2) Medium replacement: Perform the first medium replacement at 48 h, followed by subsequent replacements every 2-3 days. Cells typically reach 80-90% confluency within 2-3 days post-seeding.

3) Cell passaging protocol: Initiate subculture when cells reach 80-90% confluency. Aspirate the old medium and rinse cells with 2-3 mL PBS to remove residual serum. Add 1 mL of 0.25% Trypsin or Accutase Dissociation Reagent (Accutase is recommended; if using trypsin, strictly control digestion time). Gently rock the flask to ensure full coverage of the cell layer. For Trypsin: Aspirate excess solution after brief contact. For Accutase: Retain the solution during digestion. Incubate at 37℃ for: 1-3 min for trypsin (monitor until cell rounding is observed) or ≥5 min for Accutase. Neutralize digestion by adding 5 mL complete medium. Gently pipette to disperse the cell clusters into a single-cell suspension. Inoculate cells into new pre-coated flasks at desired ratios. Maintain culture conditions at 37℃, 5% CO_2, and saturated humidity.

Troubleshooting

Problem	Possible Cause	Solution
Low yield/low viability	Insufficient dissociation	Check the storage conditions of the digestion solution to ensure it has not been stored at 4℃ for more than 30 days
		Ensure the tissue quantity matches the kit requirements
		Ensure tissue is gently pipetted thoroughly
	Over-digestion	Strictly control the duration of the two-step digestion process
	Tissue with inadequate freshness	Accelerate tissue collection speed to prevent prolonged storage
Slow cell growth	Improper preparation of culture medium	Prepare complete culture medium with accurate ratios and avoid repeated freeze-thaw cycles
		Use complete culture medium within its validity period and avoid preparation older than three months
	Over-aged rats	Use rats at 14 days postnatal age to avoid slower proliferation and reduced passage numbers in older specimens
	Improper passaging ratio	When passaging at 1:2 ratio, calculate based on vessel surface area to maintain proper cell seeding density
	Over-passaged	Limit cell passage to 1-2 times to prevent proliferation slowdown
Low cell purity	Improper use of screening solution	Verify storage method and dosage of selection solution comply with the manufacturer's instructions
	Improper layered aspiration technique	When using separation solutions, avoid mixing upper layer tissue with lower precipitate and aspirate upper layer completely

Anatomy Images for Reference

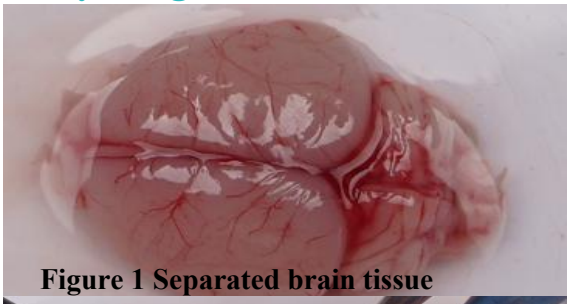


Figure 1 Separated brain tissue

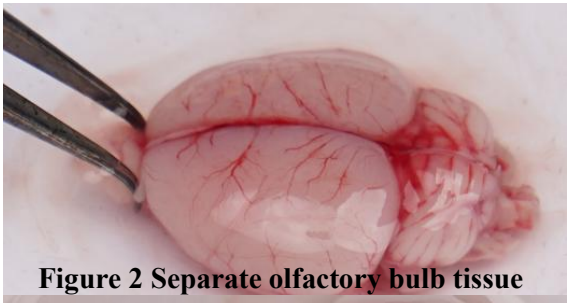


Figure 2 Separate olfactory bulb tissue

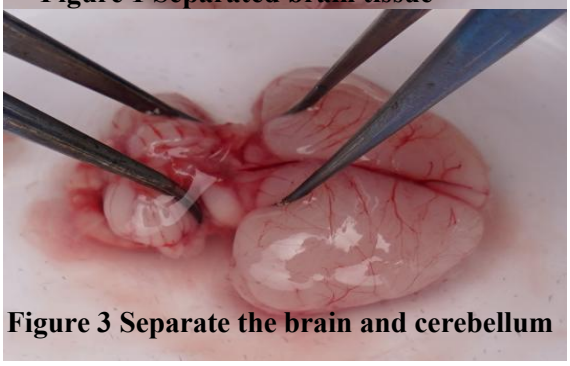


Figure 3 Separate the brain and cerebellum

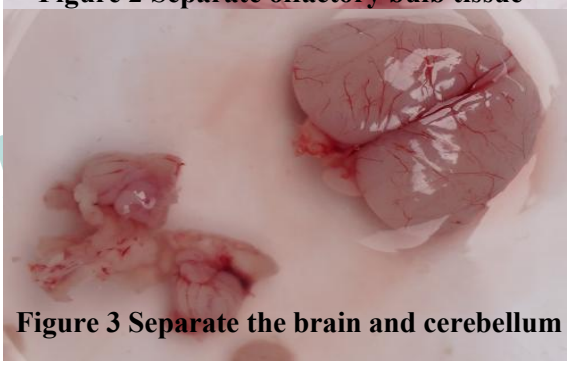


Figure 3 Separate the brain and cerebellum

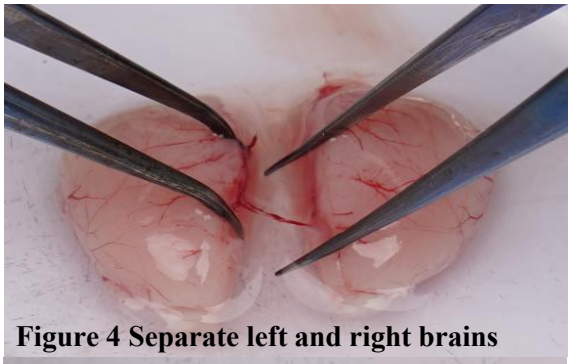


Figure 4 Separate left and right brains

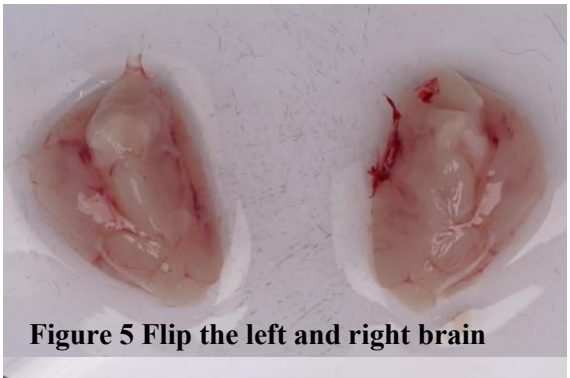


Figure 5 Flip the left and right brain

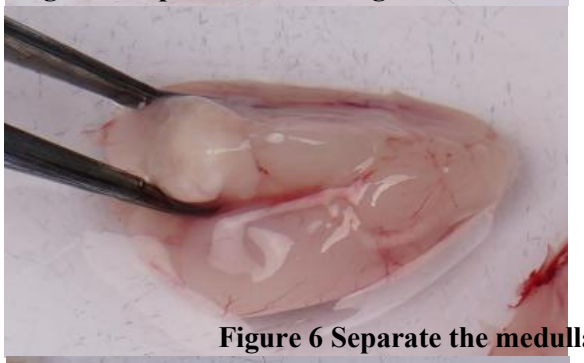




Figure 6 Separate the medulla and preserve the cerebral cortex



Figure 7 Scrape off obvious blood segments (as on the right side)

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