# Rat Brain Microvascular Pericyte Isolation and Culture Kit

**Cat. No.: P-CA-602**

**Size: 3Tests / 10Tests**

## Background

The Rat Brain Microvascular Pericyte Isolation and Culture Kit is specifically developed for the extraction of primary Rat Brain Microvascular Pericytes. 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 $1\times10^6$ cells. When passaged at a 1:2 ratio, the cells can undergo 3-5 passages. Through immunofluorescence analysis, the cell purity (α-SMA-positive rate) has been confirmed to exceed 90%.

## Scope of Application

This product is suitable for isolating Brain Microvascular Pericytes 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 Pericytes | 3Tests (250 mL)<br>10Tests (500 mL×2) | Faint Yellow Clear Liquid | 2-8°C, 1 year |
| Specialized Digestive Solution A For Rat Brain Microvascular Pericytes | 3Tests (15 mL)<br>10Tests (50 mL) | Yellow Clear Liquid | -5~-20°C, 1 year |
| Specialized Digestive Solution B For Rat Brain Microvascular Pericytes | 3Tests (1.8 mL)<br>10Tests (6 mL) | Colorless Clear Liquid | -5~-20°C, 1 year |
| Specialized Isolation Solution For Rat Brain Microvascular Pericytes | 3Tests (15 mL)<br>10Tests (50 mL) | Yellow Clear Liquid | 2-8°C, 1 year |
| Basic Culture Medium For Rat Brain Microvascular Pericytes | 3Tests (50 mL)<br>10Tests (100 mL) | Red Clear Liquid | 2-8°C, 1 year |
| Supplement For Rat Brain Microvascular Pericytes | 3Tests (10 mL)<br>10Tests (20 mL) | Yellow Clear Liquid | -5~-20°C, 1 year |
| 100 μm Cell Filter | 3Tests (3 pcs)<br>10Tests (10 pcs) | N/A | 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°C (such as Specialized Digestive Solution for Rat Brain Microvascular Pericytes) can be preserved at 4°C for 30 days after thawing. For long-term storage, aliquot them into single-use portions and freeze at -20°C. Thaw again before use to avoid repeated freeze-thaw cycles.

## Notes

1. This product is only used for scientific research or further research, not for diagnosis and treatment.
2. 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.

3. During the entire tissue dissociation process, place the small dish containing the tissue on an ice tray/ice box (2-8°C) to maintain hypothermic conditions. Critical precautions: Monitor temperature rigorously to prevent ice crystal formation in tissues/liquids.
4. 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 Pericytes; Specialized Digestive Solution B For Rat Brain Microvascular Pericytes; Supplement For Rat Brain Microvascular Pericytes: Thaw at 4°C and equilibrate to room temperature.
   ② Specialized Washing Solution For Rat Brain Microvascular Pericytes; Specialized Isolation Solution For Rat Brain Microvascular Pericytes; Basic Culture Medium For Rat Brain Microvascular Pericytes: Equilibrate to room temperature.
3) Preparation of complete culture medium: Add 10 mL of Supplement For Rat Brain Microvascular Pericytes into 50 mL of Basic Culture Medium For Rat Brain Microvascular Pericytes, mix thoroughly.

   **Note**: Complete culture medium: 2–8°C, 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°C to avoid repeated freeze-thaw cycles.

**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 Pericytes (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 Pericytes 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 Pericytes 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 Pericytes to the pellet. Mix gently and transfer the suspension to a 6 cm Petri dish. Incubate at 37°C, 5% $CO_2$ for 40 min.

② Secondary digestion: Add 0.6 mL Specialized Digestive Solution B For Rat Brain Microvascular Pericytes 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 Pericytes 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 T25 cell culture flask. Resuspend the cell pellet in 5 mL of Complete Medium For Rat Brain Microvascular Pericytes and transfer the suspension into the T25 flask. Incubate at 37°C with 5% $CO_2$ for static culture.

**Note**: Initial seeding yields approximately $2.5\times10^6$ 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: Passage should be initiated 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 solution to the flask. Tilt the flask gently to ensure even coverage of the cell monolayer. Aspirate excess trypsin, leaving a thin layer to avoid over-digestion. Place the flask in a 37°C incubator for 1-3 minutes. Monitor under an inverted microscope until >80% of cells round up and detach. Add 3-5 mL Complete Medium For Rat Brain Microvascular Pericytesto neutralize trypsin activity. Pipette gently to resuspend cells into a single-cell suspension. Transfer the cell suspension to new culture flasks at the desired split ratio. Ensure even distribution by swirling the flask. Incubate the flasks at 37°C in a humidified atmosphere with 5% $CO_2$ and saturated humidity.

**Note:** The purity of Brain Microvascular Pericytes is less than 90% when they are first plated and grown. After one passage screening, the α-SMA positivity rate identified by immunofluorescence can reach more than 90%.

## 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°C 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 subculturing 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 3-5 times to prevent proliferation slowdown |
| Low cell purity | Insufficient time of cell culture | Ensure that the cells are passaged once before identification |
| | 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

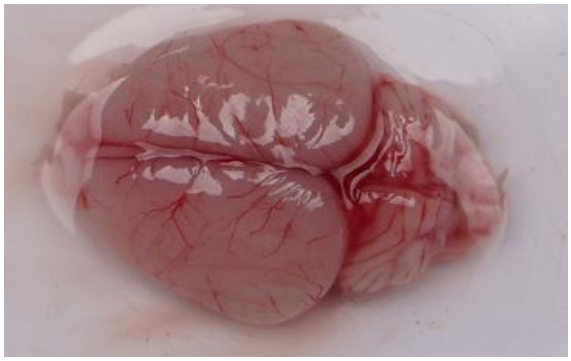


**Figure 1 Separated brain tissue**

**Figure 2 Separate olfactory bulb tissue**

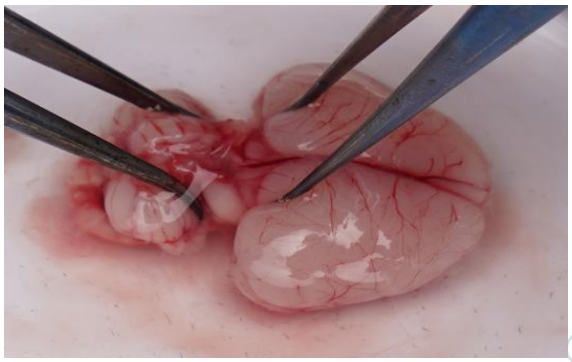


Figure 3 Separate the brain and cerebellum

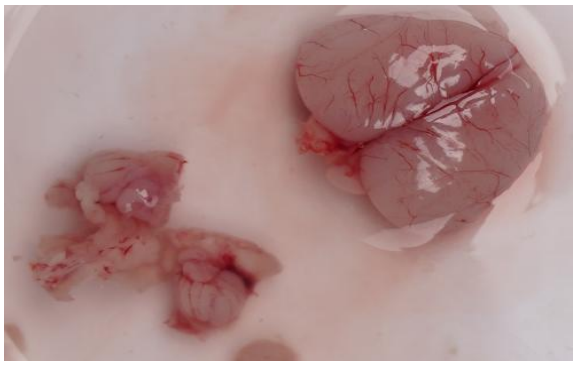


Figure 3 Separate the brain and cerebellum

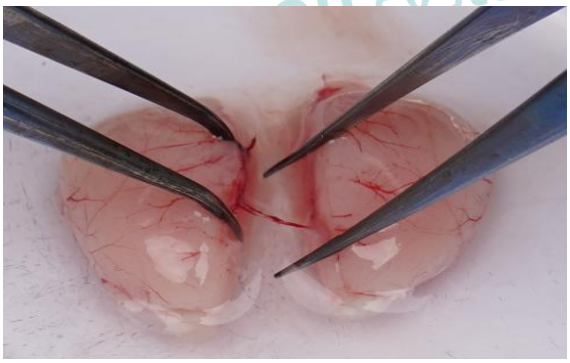


Figure 4 Separate left and right brains

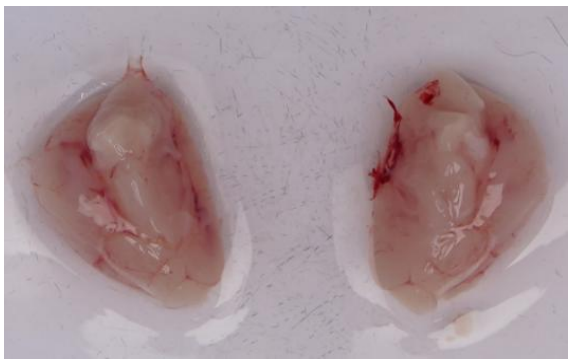
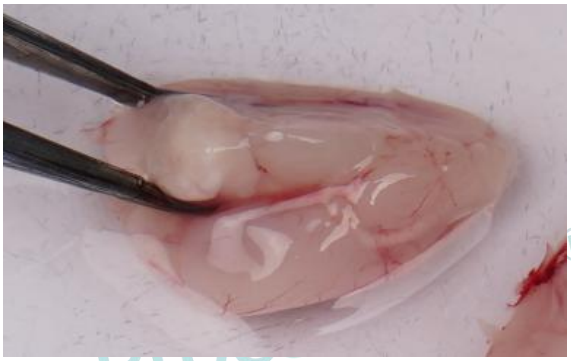


Figure 5 Flip the left and right brain



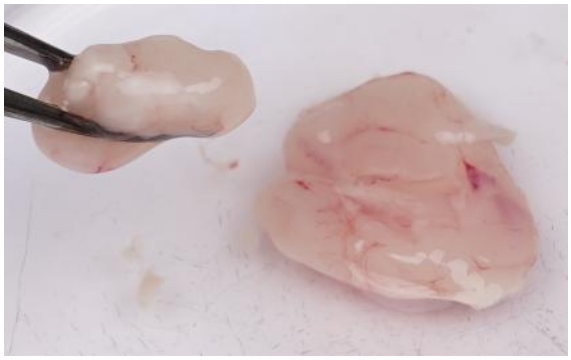


Figure 6 Separate the medulla and preserve the cerebral cortex

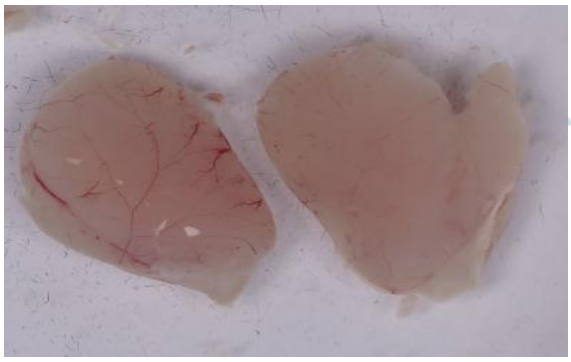


Figure 7 Scrape off obvious blood segments (as on the right side)