Rat Brain Artery Vascular Smooth Muscle Cell Isolation and Culture Kit

Cat. No. : P-CA-611

Size : 3Tests / 10Tests

Background

The Rat Brain Artery Vascular Smooth Muscle Cell Isolation and Culture Kit is specifically developed for the extraction of primary rat brain artery vascular smooth muscle cells. Verified through standardized procedures, each 1 Test of this kit enables the acquisition of one flask of cells (T-25 culture flask), with a cell count exceeding 1×10^6 cells. When subcultured at a 1:2 ratio, the cells can undergo 5 passages, with the best cell state within the first 3 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 Brain Artery Vascular Smooth Muscle Cells from various rat strains (e.g.,Wistar and SD) aged 35-42-days . After processes of tissue isolation, enzymatic digestion, and 5 days planting, a yield of > 1×10^6 cells can be obtained.

Note: The intact saphenous vein tissue extracted from 10 rats (with tissue yield of each hind limb shown in Figure 4) typically yield enough cells for one T-25 flask..The exact number of rats required may vary depending on the size and quantity of brain artery vascular tissue harvested during the procedure. If the amount of tissue obtained is small, the number of experimental rats can be increased appropriately to avoid insufficient cell quantity.

Kit Components

Name	Size	Appearance	Storage and Expiration Date
Specialized Washing Solution For Rat Brain Artery Vascular Smooth Muscle Cells	3Tests (250 mL) 10Test (500 mL×2)	Pale Yellow Clear Liquid	2-8°C,1 year
Specialized Digestive Solution For Rat Brain Artery Vascular Smooth Muscle Cells	3Tests (15 mL) 10Tests (50 mL)	Yellow Clear Liquid	-5~-20°C,1 year
Basic Culture Medium For Rat Brain Artery Vascular Smooth Muscle Cells	3Tests (50 mL) 10Tests (100 mL)	Red Clear Liquid	2-8°C,1 year
Supplement For Rat Brain Artery Vascular Smooth Muscle Cells	3Tests (5 mL) 10Tests (10 mL)	Yellow Clear Liquid	-5~-20°C,1 year

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 Artery Vascular Smooth Muscle Cells) can be preserved at 4℃ for 30 days after thawing. For long-term storage, aliquot them into single-use portions and store at -20℃. Thaw again at 4°C 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. Reagent preparation or aliquoting 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; ; Phosphate-Buffered Saline (PBS); surgical instruments (At least 2 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; 1 pair of micro scissors); 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 For Rat Brain Artery Vascular Smooth Muscle Cells; Supplement For Rat Brain Artery Vascular Smooth Muscle Cells: Thaw at 4°C and equilibrate to room temperature.
   ② Specialized Washing Solution For Rat Brain Artery Vascular Smooth Muscle Cells; Basic Culture Medium For Rat Brain Artery Vascular Smooth Muscle Cells: Equilibrate to room temperature.
3) Preparation of complete culture medium: Add 5 mL of Supplement For Rat Brain Artery Vascular Smooth Muscle Cells into 50 mL of Basic Culture Medium For Rat Brain Artery Vascular Smooth Muscle Cells, mix thoroughly.
   Note: Storage conditions for 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 -20℃ to avoid repeated freeze-thaw cycles.

2. Dissection Protocol

1) Animal Sterilization and Euthanasia: Perform euthanasia via pentobarbital sodium overdose injection, 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; 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: Cut the skin to expose the entire hind leg, pay attention to keep hair pulled away from the anatomical area to prevent contamination.
      b. Use Straight Forceps 1 to clamp the mouth of the rat vertically and fix it, use the Ophthalmic Scissors 2 to cut the cervical vertebrae from the neck, and use the 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 Artery Vascular Smooth Muscle Cells (Figure 1).

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.

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 Under a dissection microscope, place a culture dish containing 10 mL of Specialized Washing Solution For Rat Brain Artery Vascular Smooth Muscle Cells. Place a brain tissue into the dish with the hypothalamus side facing upwards.

③ Using the left hand, find the lower end of the basilar artery in the basilar artery sulcus with a fine forceps. Then, lift the basilar artery with the fine forceps, which will also pull up the dura mater covering it. Using the right hand, carefully use a microscissors to separate the artery from the dura mater while lifting it, performing the dissection step-by-step (Figure 2).

④ Segmental isolation of cerebral arteries is generally divided into six segments: the basilar artery and its connected superior cerebellar artery (①), bilateral posterior cerebral arteries (②), bilateral middle cerebral arteries (③), and the anterior cerebral artery (④) (Figure 3). Place the isolated cerebral arteries in a new petri dish containing 3 mL of Specialized Washing Solution For Rat Brain Artery Vascular Smooth Muscle Cells for further use.

⑤ Under the dissection microscope, use the left hand to hold the straight micro forceps and the right hand to hold the curved micro forceps to remove the dura mater, brain parenchyma, and other tissues attached to the cerebral artery (Figure 4), leaving only the pure cerebral artery vascular tissue. Then, place it into a new petri dish containing 5 mL of Specialized Washing Solution for Rat Brain Artery Vascular Smooth Muscle Cells and rinse once.

2) Tissue Digestion

① Put the pure cerebral artery vascular tissue into a 6cm culture dish containing 5mL Specialized Digestive Solution For Rat Brain Artery Vascular Smooth Muscle Cells. Use the Micro Straight Forceps in the left hand to clamp the tissue, and use the Micro Scissors in the right hand to cut the tissue into 5mm^2 fragments , Place the petri dish in the 37°C incubator and digested overnight for 16-18 hours.

② After digestion, remove the petri dish from the incubator and use a 5 mL pipette or a Pasteur pipette to blow the suspension about 30 times to disperse the large tissue visible to the naked eye. After mixing, add 5 mL Specialized Washing Solution For Rat Brain Artery Vascular Smooth Muscle Cells to the petri dish.

Note: The digestion time should be adjusted based on the actual digestion effect, which can be observed under a microscope. Well-digested tissue fragments will exhibit distinct rounded cell arrangements, with some foating cells present in the digestive solution. After pipetting to mix, a small number of cell clusters and debries may remain, which is a normal occurence.

3) Cell Isolation

① Transfer the cell suspension to a 15mL centrifuge tube and centrifuge at 1200rpm for 5min. Discard the supernatant and retain the precipitate.

4. Cell Culture and Subculture

1) Cell seeding: Take out the T25 cell culture flask, and resuspend the cell pellet in the centrifuge tube with 5 mL Complete Culture Medium For Rat Brain Artery Vascular Smooth Muscle Cells, and inoculated into the T25 cell culture flask. The cells were cultured in a incubator at 37°C,5% CO_2
2) Medium replacement: Perform the first medium change at 48 h, followed by subsequent replacements every 2-3 days. Cells typically reach 80-90% confluency within 5-7 days post-seeding.
3) Cell Subculture: When the cell confluence reaches 80-90%, it is ready for passage. First, aspirate and discard the medium from the T25 cell culture flask and wash the cells once with 2-3 mL PBS. Then, add 1 mL of 0.25% trypsin digestion solution to the T25 flask, gently rotate the flask until the digestion solution covers the entire bottom of the flask, then aspirate any excess trypsin solution, and incubate at 37°C for 1-3 min. Observe under an inverted microscope until the cells retract and become rounded, then add 5 mL of Complete Medium for Rat Brain Artery Vascular Smooth Muscle Cellsto terminate the digestion, gently resuspend and disperse the cells, Mix the precipitate gently with a 5 mL pipette or Bacto pipette, and depending on the passage ratio or experimental requirements, inoculate the cells into new culture vessels and incubate them statically in a cell culture incubator at 37°C,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°C for more than 30 days
		Ensure the tissue quantity matches the kit requirements
		Make sure the tissue is not cut too large
		Ensure that the tissue is gently and adequately pipetted up and down
	Over-digestion	Strictly control the size of the tissue block to avoid cutting it too small
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 35-42 days postnatally to prevent slower cell proliferation and reduced passage numbers associated with cells extracted from older rats.
	Improper subculturing ratio	When passaging at 1:2 ratio, calculate based on the vessel surface area to maintain proper cell seeding density
	Over-passaged	Limit cell passage to 3-5 times to prevent a slowdown in proliferation.
	shortage of tissue sampling amount	If the tissue amount of the great brain artery vessel is small, the rat amount can be increased appropriately
Low cell purity	The outer membrane layer of the tissue was not completely removed	Ensure that the outer membrane and cerebral cortex tissue are completely removed

Anatomy Images for Reference

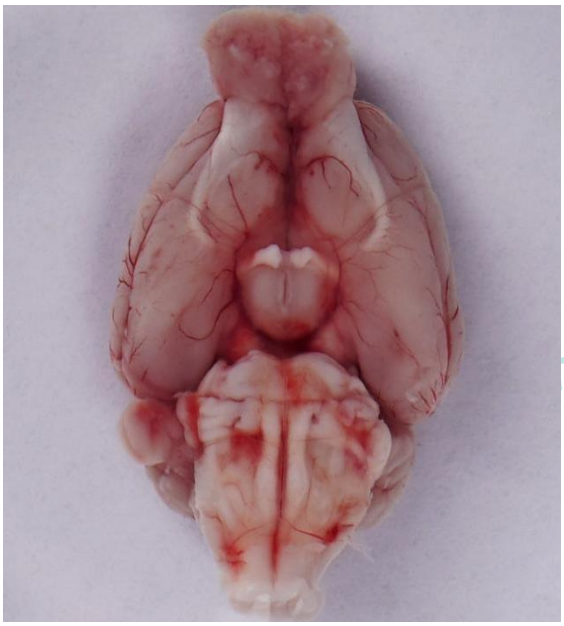
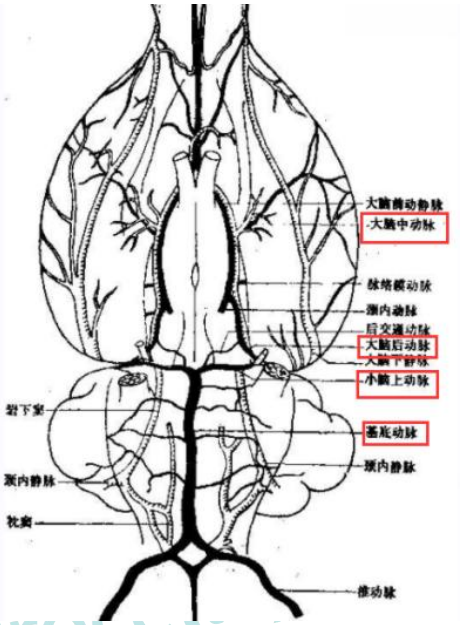


Figure 1 The complete brain and cerebellum tissue were separated



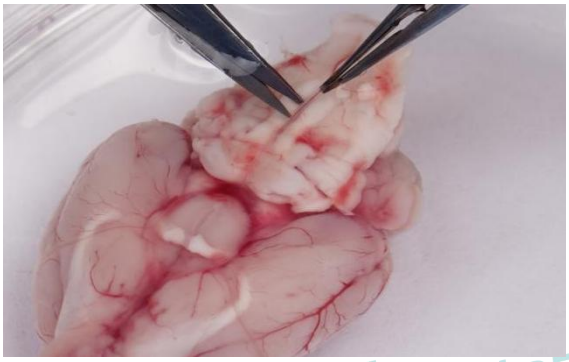


Figure 2 Sever the basilar artery and its associated superior cerebellar artery

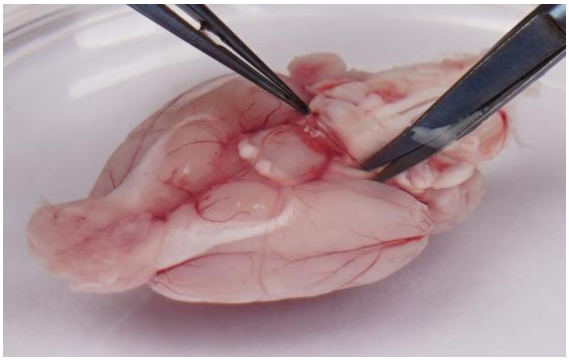


Figure 3 Decompression of bilateral posterior cerebral arteries

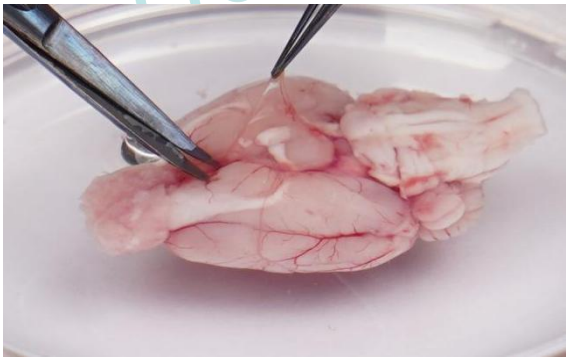


Figure 3 Decompression of bilateral middle cerebral arteries

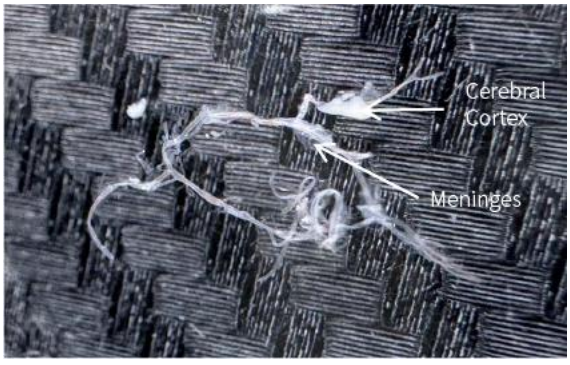


Figure 4 removed cerebral arterial vascular tissue