Rat Saphenous Vein Smooth Muscle Cell Isolation and Culture Kit

Cat. No. : P-CA-610

Size : 3Tests / 10Tests

Background

The Rat Saphenous Vein Smooth Muscle Cell Isolation and Culture Kit is specifically developed for the extraction of primary rat saphenous vein 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 extracting Saphenous Vein Smooth Muscle Cells from various rat strains (e.g., Wistar, SD) aged 20-30 days. After processes of tissue isolation, enzymatic digestion, and 48-hour plating, a yield of $>1\times10^6$ cells can be obtained.

Note: The intact saphenous vein tissue extracted from 7 rats (with tissue yield of each hind limb shown in Figure 5) typically yield enough cells for one T-25 flask. The exact number of rats required may vary depending on the size and quantity of saphenous vein tissue harvested during this procedure. If the amount of tissue obtained is insufficient, additional experimental rats may be needed to prevent cell quantity deficiency.

Kit Components

Name	Size	Appearance	Storage and Expiration Date
Specialized Washing Solution For Rat Saphenous Vein Smooth Muscle Cells	3Tests (250 mL) 10Test (500 mL×2)	Pale Yellow Clear Liquid	2-8°C,1 year
Specialized Digestive Solution For Rat Saphenous Vein Smooth Muscle Cells	3Tests (15 mL) 10Tests (50 mL)	Yellow Clear Liquid	-5~-20°C,1 year
Basic Culture Medium For Rat Saphenous Vein Smooth Muscle Cells	3Tests (50 mL) 10Tests (100 mL)	Red Clear Liquid	2-8°C,1 year
Supplement For Rat Saphenous Vein 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°C (such as Specialized Digestive Solution for Rat Saphenous Vein Smooth Muscle Cells) can be preserved at 4°C for 30 days after thawing. For long-term storage, aliquot them into single-use portions and store at -20°C. 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 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 Preparations

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, 1 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 flasks, dissection board (foam board can substitute) and multiple 2 mL/15 mL/50 mL centrifuge tubes.
2) Reagent Thawing and Rewarming:
   ① Specialized Digestive Solution for Rat Saphenous Vein Smooth Muscle Cells & Supplement for Rat Saphenous Vein Smooth Muscle Cells: Thaw at 4°C and equilibrate to room temperature.
   ② Specialized Washing Solution for Rat Saphenous Vein Smooth Muscle Cells & Basic Culture Medium for Rat Saphenous Vein Smooth Muscle Cells: Equilibrate to room temperature.
3) Preparation of Complete Culture Medium: Add 5 mL of Supplement for Rat Saphenous Vein Smooth Muscle Cells into 50 mL of Basic Culture Medium for Rat Saphenous Vein Smooth Muscle Cells, mix thoroughly.

   Note: Storage conditions for complete culture medium: 2–8℃, valid for 3 months. When preparing the complete culture medium, it can be prepared according to the usage amount. Remaining supplement should be aliquoted proportionally and stored at -20℃ to avoid repeated freeze-thaw cycles.

2. Dissection Procedures

1) Animal Sterilization and Euthanasia: Perform euthanasia via pentobarbital sodium overdose injection or cervical dislocation, then immerse the carcass 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 in order of use from left to right on two sterilized EP tube racks (Ophthalmic Scissors 1 and Straight Forceps , Ophthalmic Scissors 2 and Curved Forceps).

   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 supine position within the clean bench using needles, preparing for tissue harvesting.
   ③ Tissue Harvesting Procedure:
      a. Using Straight Forceps to grasp the ankle skin of the hind leg, cut the skin bilaterally from bottom to top with Ophthalmic Scissors 1, and cut up to the abdomen.

      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. Find the largest and most prominent great saphenous vein on the inner thigh, then use Curved Forceps in the left hand to clamp the great saphenous vein on the ankle, and use the Ophthalmic Scissors 2 in the right hand to carefully cut the great saphenous vein tissue with attached muscles. The tissue was placed in a glass dish containing 10ml of Specialized Washing Solution for Rat Saphenous Vein Smooth Muscle Cells.

      Note: Cut the skin to expose the entire hind leg, pay attention to keep hair pulled away from the anatomical area to prevent contamination.

3. Tissue Processing and Digestion

1) Tissue Processing
   ① Put Micro Straight Forceps, Micro Curved Forceps and Micro Scissors on the EP tube rack

within the clean bench, ensuring the distal third of each tool suspended.

② Tissue dissection with this set of new micro scissors and forceps. Rinse the tissue once and place it into a new petri dish containing 10 ml of Specialized Washing Solution for Rat Saphenous Vein Smooth Muscle Cells.

③ Saphenous Vein Tissue Dissection:

a. Fix the thicker end of the vascular tissue with Micro Straight Forceps in the left hand, whlie carefully tear apart the muscular and connective layers of the vascular adventitia using Micro Curved Forceps in the right hand. Find a white, independently growing saphenous nerve (Figure 1), and use Micro Curved Forceps to pull out and discard the saphenous nerve.

b. Fix the vascular tissue with Micro Straight Forceps in the left hand, while carefully remove the muscle and connective tissues surrounding the vascular tissue using Micro Curved Forceps in the right hand (Figure 2). Obtain clean and intact vascular tissue (Figure 3). Separate the saphenous vein and artery that are growing together, remove the arterial tissue, and trim the obvious adventitia (Figure 4).

④ Tissue fragmentation: Hold the thicker end of the great saphenous vein with Micro Straight Forceps in the left hand, and insert one blade of the Micro Scissors into the lumen of the great saphenous vein with the right hand to attempt a longitudinal dissection of the vein tissue (Figure5). Transfer the tissue to a new petri dish containing 10 ml of Specialized Washing Solution for Rat Saphenous Vein Smooth Muscle Cells.

Note: The great saphenous vein is accompanied by the artery, appearing thicker than arteries and darker in color, and has some vascular branches. Care should be taken to distinguish it from the smooth saphenous nerve. During procedures, avoid excessive stretching of tissues to prevent cellular damage. Repeated washing is recommended to avoid contamination, as tissues may come into contact with hair.

2) Tissue Digestion

① Place the purified saphenous vein tissue into a 6cm culture dish containing 5mL Specialized Digestive Solution for Rat Saphenous Vein 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 (Figure6). 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 Pipette the suspension approximately 30 times to disperse the large tissue visible to the naked eye. After mixing thoroughly, add 5 mL of Specialized Washing Solution for Rat Saphenous Vein 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 (Figure 7), 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 cell pellet.

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 of Complete Culture Medium of Rat Saphenous Vein Smooth Muscle Cells, then inoculat into the T25 cell culture flask. The cells were cultured in a incubator at 37°C, 5% CO_2.

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

2) Medium Renewal: Perform the first medium change 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 Subculture: When the cell confluence reaches 80-90%, it is ready for passaging. First, aspirate and discard the medium from the T25 cell culture flask and wash the cells once with 2-3 mL of PBS. Then, add 1mL of 0.25% trypsin digestive solution to the T25 flask, gently rotate the flask until the digestive solution covers the entire bottom, then aspirate and disgard the excess trypsin solution, incubate at 37°C for 1-3 min. Next, observe under an inverted microscope until the cells retract and become rounded, then add 5mL of Complete Culture Medium for Rat Saphenous Vein Smooth Muscle Cells to terminate the digestion. Resuspend and disperse the cells by gently pipetting with a 5 mL pipette or Pasteur pipette. Inoculate the cells into new culture vessels according to the split ratio or experimental requirements. 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 smooth muscle 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 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 aged 20-30 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 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 great saphenous vein 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 is completely removed

Anatomy Images for Reference

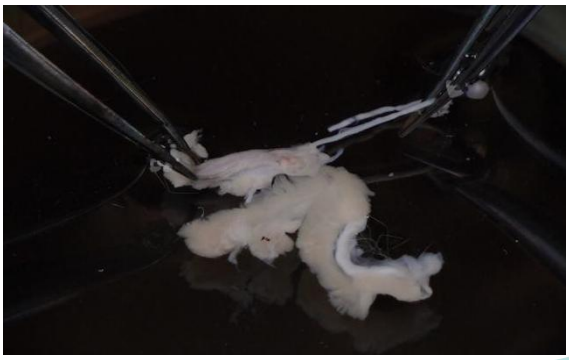

Figure 1a. Incise the muscular and connective tissues, and excise the hidden nerve.

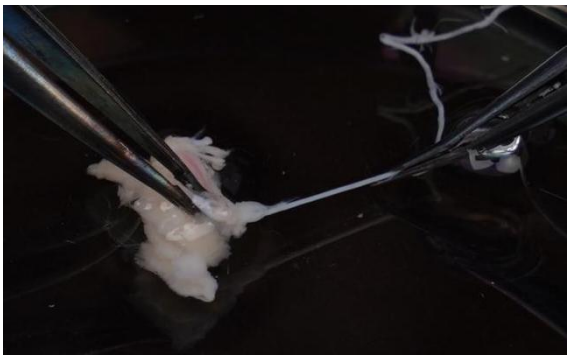

Figure 1b. Incise the muscular and connective tissues, and excise the hidden nerve.

Figure 2a. Clearance of muscular and connective tissues.

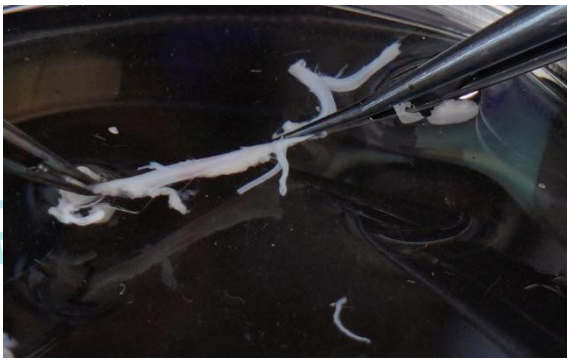

Figure 2b. Clearance of muscular and connective tissues.

Figure 3. Obtain clean and intact vascular tissue.

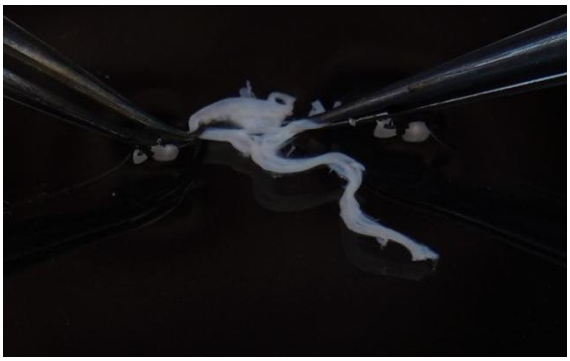

Figure 4. Separate the saphenous vein and artery that are growing together.

Figure 5. Attempt a longitudinal dissection of the vein tissue.

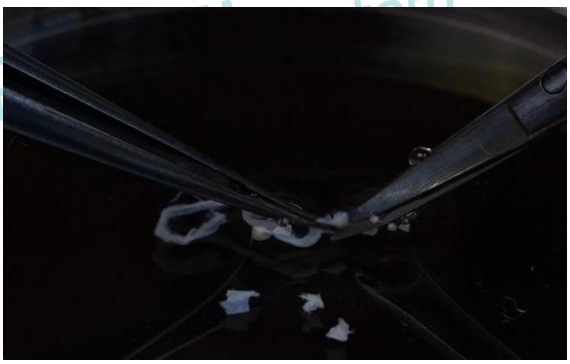

Figure 6a. Cut the saphenous vein into fragments.

Figure 6b. Cut the saphenous vein into fragments.

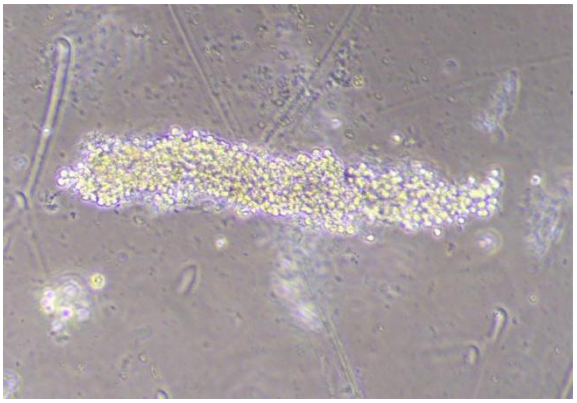

Figure 7. Microscopic observation of well digested tissue.