Rat Aortic Smooth Muscle Cell Isolation and Culture Kit

Cat. No. : P-CA-607
Size : 3Tests / 10Tests

Background

The Rat Aortic Smooth Muscle Cell Isolation and Culture Kit is specifically developed for the extraction of primary rat Aortic Smooth Muscle 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 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 Aortic Smooth Muscle Cells from 20-30-day-old rats of various strains,such as Wistar and SD. Through processes of tissue isolation, digestion, and 48-hour planting purification, a yield of $>1\times10^6$ cells can be obtained.

Note: The extraction of intact aorta pectoralis tissue from 5 rats is required to yield sufficient cells for one T-25 flask(The amount of aortic tissue obtained by each rat is shown in Figure 3). The exact number of rats required may vary depending on the size and quantity of aorta pectoralis 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 Aortic Smooth Muscle Cells	3Tests (250 mL) 10Test (500 mL×2)	Faint Yellow Clear Liquid	2-8°C,1 year
Specialized Digestive Solution For Rat Aortic Smooth Muscle Cells	3Tests (15 mL) 10Tests (50 mL)	Yellow Clear Liquid	-5~-20°C,1 year
Basic Culture Medium For Rat Aortic Smooth Muscle Cells	3Tests (50 mL) 10Tests (100 mL)	Red Clear Liquid	2-8°C,1 year
Supplement For Rat Aortic 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 Aortic 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 Preparation

1) Self-supplied Reagents and Consumables: two Eppendorf (EP) tube racks;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; 1 pair of micro scissors); 6 cm/10 cm culture dishes; T25 culture flask; dissection board (Foam board can be used as a substitute): assorted 2 mL/15 mL/50 mL centrifuge tubes.
2) Reagent Thawing and Rewarming:
   ① Specialized Digestive Solution For Rat Aortic Smooth Muscle Cells; Supplement For Rat Aortic Smooth Muscle Cells: Thaw at 4°C and equilibrate to room temperature.
   ② Specialized Washing Solution For Rat Aortic Smooth Muscle Cells; Basic Culture Medium For Rat Aortic Smooth Muscle Cells: Equilibrate to room temperature.
3) Preparation of Complete Culture Medium: Add 5 mL of Supplement For Rat Aortic Smooth Muscle Cells into 50 mL of Basic Culture Medium For Rat Aortic Smooth Muscle Cells, mix thoroughly.

Note: Storage conditions for complete culture medium: 2-8°C, 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°C to avoid repeated freeze-thaw cycles.

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; 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 supine position within the clean bench using needles for stabilization during tissue harvesting.
   ③ Tissue Sampling:
      a. Using Straight Forceps 1 to grasp the upper abdominal skin, cut the skin from the upper abdomen to the neck, and cut the skin on both sides upward to the neck using Ophthalmic Scissors 1.Until the sternum is fully exposed.

         Note: Cut the skin to expose the entire chest, taking care to tear the hair away from the anatomical area to prevent contamination.

      b. Hold the right inferior rib arch of the rat with Curved Forceps 2, and use Ophthalmic Scissors 2 to cut upward from the rib to the clavicle. Cut horizontally through the diaphragm, and cut along the left inferior rib to the left shoulder clavicle area. Cut the sternal handle, flip it upward, and fully open the thoracic cavity to expose the heart and lung tissues.

         Note: Do not insert the scissors too deep into the thoracic cavity. Cut lightly upward and forward, and do not cutting through lung tissue can easily cause contamination

      c. Use curved forceps 3 to push aside the lung tissue to the left, exposing the aortic tissue. Pay attention to distinguishing between the esophagus and the aorta. The aorta grows closely against the thoracic vertebrae, appearing grayish-white with blood inside; the

esophagus grows independently, yellowish-white. Use Curved Forceps 3 to clamp the aorta, and use ophthalmic Scissors 3 to dissect the aorta away from the thoracic vertebra. Use Ophthalmic Scissors 3 to cut along the aortic arch and at the junction with the abdominal aorta where it meets the diaphragm, obtaining a complete segment of thoracic aortic tissue. Place this tissue in a culture dish containing 10mL of Specialized Washing Solution For Rat Aortic Smooth Muscle Cells.（Figure 1）

Note: Only the first set of instruments may contact external skin; Other instruments are strictly prohibited from touchingexternal skin and hair. If they do, sterile instruments must be replaced to prevent contamination.

2. 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 Aortic tissue purification: Tissue purification with the new micro forceps set. The left hand uses Micro Straight Forceps to fix one end of the aortic tissue, and the right hand uses Micro Curved Forceps to hold the aortic tissue, and gently tear off the yellow and pink fat and connective tissue of the adventitia. (Figure 2). Clean and put it into a culture dish containing 10mL Specialized Washing Solution For Rat Aortic Smooth Muscle Cells (Figure 3).

③ Tissue fragmentation: The left hand uses Micro Straight Forceps to clamp one end of the aortic tissue, and the right hand inserts a blade of Micro Scissors into the aortic blood vessel to cut the aortic tissue longitudinally (Figure 4).Put the tissue in a culture dish containing 10mL Specialized Washing Solution For Rat Aortic Smooth Muscle Cells.The left hand use Micro Straight Forceps to fix one end of the aorta.The inner membrane was facing up, and the back of the Micro Curved Forceps hook was used to gently scrape the inner membrane surface dozens of times with the right hand to remove the endothelial cells.Subsequently, the smooth muscle layer is scraped off in sheets using the back of the Micro Curved Forceps(Figure 5), and the adventitia layer is removed.

2) Tissue Digestion

① Put the pure middle layer smooth muscle tissue of the aorta into a 6cm culture dish containing 5mL Specialized Digestive Solution For Rat Aortic 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 small pieces of about 5mm^2 (Figure 6), The petri dish was placed 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 Aortic Smooth Muscle Cells to the petri dish.

Note: The digestion time is determined according to the actual digestion effect. It can be observed under the microscope. There will be obvious circular cell arrangement on the freshly digested tissue block (Figure 7), and some cells will be free in the digestion solution. A small number of cell clusters and fragments will still be present after mixing with blowing, which is a normal phenomenon.

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.

3. Cell Culture and Subculture

1) Cell seeding: The T25 cell culture flask was taken out, and the precipitate in the centrifuge tube

was resuspended with 5 mL rat aortic smooth muscle cell complete culture medium, 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 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) Subculture protocol: When the cell confluence reaches 80-90%, it is ready for passage. First, aspirate the medium from the T25 cell culture flask and wash the cells once with 2-3 mL PBS of water; add 1mL 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 5mL of rat aortic smooth muscle complete medium to terminate the digestion, gently resuspend and disperse the cells, centrifuge at 1200 rpm for 5 min, discard the supernatant, and add rat aortic endothelial cell complete medium. 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 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 at 20-30 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
	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
	The endothelial cells were not completely destroyed	Make sure all areas of the inner lining are scraped back and forth dozens of times

Anatomy Images for Reference

Figure 1. The separated complete thoracic aorta tissue.

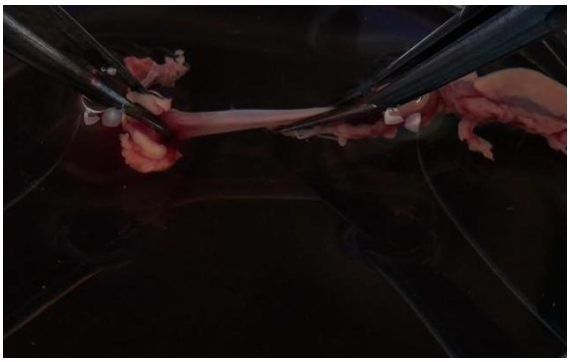

Figure 2a. Clear the adventitia fat and connective tissue of the aorta.

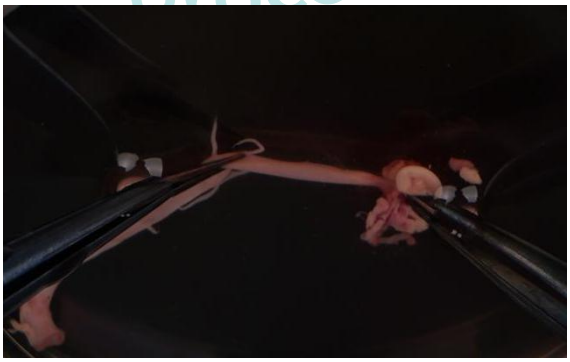

Figure 2b. Clear the adventitia fat and connective tissue of the aorta.

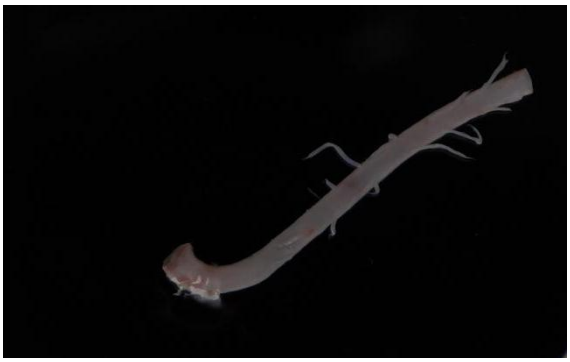

Figure 3. Pure aortic tissue

Figure 4a. Longitudinally section the aortic tissue

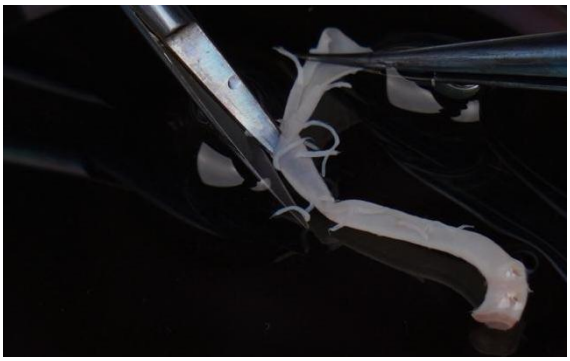

Figure 4b. Longitudinally section the aortic tissue

Figure 5a. The aortic smooth muscle layer was scraped and the outer membrane layer was removed

Figure 5b. The aortic smooth muscle layer was scraped and the outer membrane layer was removed

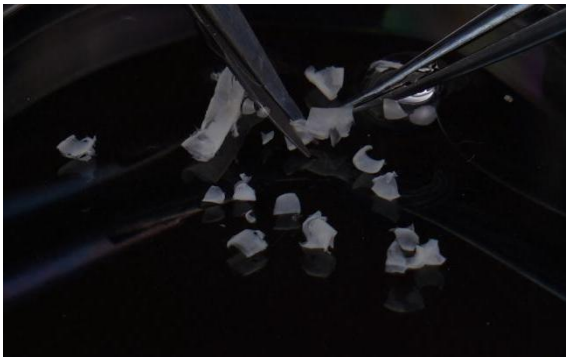

Figure 6a. Chop the aortic smooth muscle tissue into pieces.

Figure 6b.Chop the aortic smooth muscle tissue into pieces.

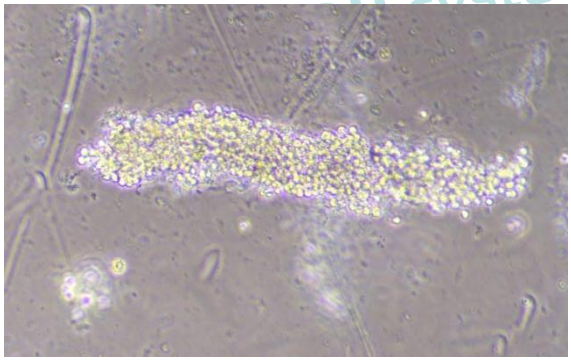

Figure 7. Microscopic observation of well digested tissue

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