# Rat Aortic Endothelial Cell Isolation and Culture Kit

**Cat. No.: P-CA-606**

**Size: 3Tests / 10Tests**

## Background

The Rat Aortic Endothelial Cell Isolation and Culture Kit is specifically developed for the extraction of primary Rat Aortic 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 $1\times10^6$ cells. When passaged at a 1:2 ratio, the cells can undergo 2-3 passages.Through immunofluorescence analysis, the cell purity (CD31-positive rate) has been confirmed to exceed 90%.

## Scope of Application

This product is suitable for extracting Aortic Endothelial Cells from rats aged 20-30 days of various strains,such as Wistar and SD. Through processes of tissue isolation, digestion, and 72-hour planting purification, a yield of $>1\times10^6$ cells can be obtained.

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

## 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. 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 (including 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) and assorted 2 mL/15 mL/50 mL centrifuge tubes.
2) Reagent Thawing and Rewarming:
   ① Specialized Digestive Solution For Rat Aortic Endothelial Cells, Supplement For Rat Aortic Endothelial Cells: Thaw at 4°C and equilibrate to room temperature.
   ② Specialized Washing Solution For Rat Aortic Endothelial Cells, Basic Culture Medium For Rat Aortic Endothelial Cells: Equilibrate to room temperature.
3) Preparation of Complete Culture Medium: Add 5 mL of Supplement For Rat Aortic Endothelial Cells into 50 mL of Basic Culture Medium For Rat Aortic Endothelial 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.**
4) Coating of Culture Vessels: Add 1 mL of Planting Solution For Rat Aortic Endothelial Cells into a T25 culture flask. Gently swirl to ensure even coverage of the bottom surface. Incubate the flask in a 37°C, 5% $CO_2$ incubator for 0.5-2 hours.

**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 from left to right on two sterilized EP tube racks (Ophthalmic Scissors 1 and Straight Forceps 1, Ophthalmic Scissors 2 and Curved Forceps 1, Ophthalmic Scissors 3 and Curved Forceps 2).
   **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-contamination.**
   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, then 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, ensuring 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 1, 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: Avoid inserting the scissors too deeply into the thoracic cavity. Gently cut upwards and forwards, ensuring not to cut through the lung tissue, as this can easily lead to contamination.**

c. Use curved forceps 2 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 2 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 10 mL of Specialized Washing Solution For Rat Aortic Endothelial Cells (Figure 1).

**Note: Throughout the entire tissue sampling procedure, only the first set of scissors and forceps are permitted to contact the external skin of the rat. Other instruments are strictly prohibited from touching the external skin or fur. If any contact occurs, sterile instruments must be replaced 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 purification with this new set of micro scissors and forceps. Use Micro Straight Forceps in the left hand to fix one end of the aortic tissue, while using Micro Curved Forceps in the right hand to hold the aortic tissue, gently tear off the yellow-pink adipose and connective tissue of the adventitia (Figure 2). Clean and put it into a culture dish containing 10 mL of Specialized Washing Solution For Rat Aortic Endothelial Cells (Figure 3).

③ Use Micro Straight Forceps in the left hand to clamp one end of the aortic tissue, and insert a blade of Micro Scissors into the aortic blood vessel with the right hand to cut the aortic tissue longitudinally (Figure 4). Put the tissue in a culture dish containing 10 mL of Specialized Washing Solution For Rat Aortic Endothelial Cells.

2) Tissue Digestion

① Place the aortic tissue into a 6cm culture dish containing 5 mL of Specialized Digestive Solution For Rat Aortic Endothelial 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 approximately 1 cm in length. The petri dish is then placed in a 37°C incubator and digested for 30 minutes.

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 detach the endothelial cells. After mixing thoroughly, use Micro Curved Forceps to remove and discard the large chunks of aortic tissue, add 5 mL of Specialized Washing Solution For Rat Aortic Endothelial Cells to the petri dish.

3) Cell Isolation

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

② Slowly add the digested suspension to the filter using a pipette. Collect filtrate in the 50 mL tube. Rinse the filter with 2 mL of washing solution to maximize cell collection.

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

③ Transfer the cell suspension to a 15 mL centrifuge tube and centrifuge at 1200 rpm for 5 min. Discard the supernatant and retain the 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 Aortic Endothelial Cells. Slowly add 5mL of Specialized Washing Solution For Rat Aortic Endothelial Cells along the inner wall of the flask to avoid disrupting the coated surface. Aspirate the washing solution after rinsing gentlely. Resuspend the pellet in the centrifuge tube with 5 mL of Complete Culture Medium for Rat Aortic Endothelial Cells. Then inoculate cell suspension into the T25 cell culture flask. The cells were cultured in an incubator at 37°C, 5% $CO_2$.
2) Medium Renewal: Conduct the first medium change via centrifugation at 48 h, followed by subsequent replacements every 2-3 days. Cells typically reach 80-90% confluency within 5-7 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 of PBS. 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, 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 Culture Medium for Rat Aortic Endothelial Cells to terminate the digestion. Gently resuspend and disperse the cells, 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 |
| | | 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 the complete culture medium within its validity period and avoid preparation exceeding 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 the vessel surface area to maintain proper cell seeding density |
| | Over-passaged | Limit cell passage to 2-3 times to prevent proliferation slowdown |
| | Shortage of tissue sampling amount | If the tissue amount of aortic is small, the rat amount can be increased appropriately |
| Low cell purity | The outer membrane and adipose tissue were not completely removed | Make sure the blood vessels are cleaned out |
| | The tissue block is too small | If the tissue block is small, the digestion time can be shortened by 5-10 min |

## Anatomy Images for Reference



**Figure 1. The separated complete thoracic aorta tissue。**

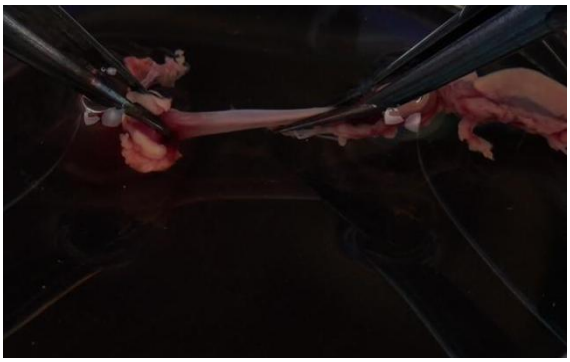


**Figure 2a. Clear the adventitia adipose and connective tissue of the aorta.**



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

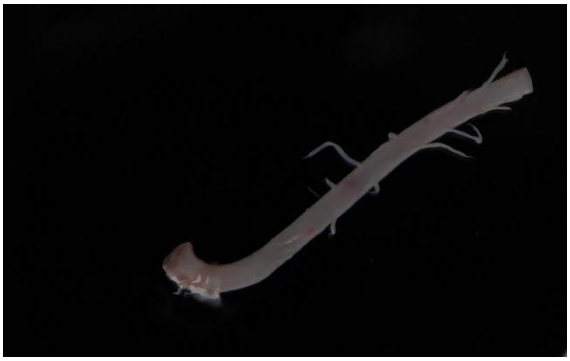


**Figure 3. Pure aortic tissue**



**Figure 4a. Longitudinally section the aortic tissue**



**Figure 4b. Longitudinally section the aortic tissue**

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