Rat Cardiac Microvascular Endothelial Cell Isolation and Culture Kit

Cat.No. : P-CA-620

Size：3Tests / 10Tests

Background

This kit is developed specifically for the isolation of various primary rat cardiac microvascular endothelial cells. The tissue is digested using a two-step digestion method, and the cells are purified by density gradient centrifugation. After laboratory verification, it can isolate more than 1×10^6 cells, and the immunofluorescence identification (CD31 positive rate) more than 90%.

Scope of Application

This product is suitable for extracting cardiac microvascular endothelial cells from different strains of rats such as Wistar and SD at 15-20 days of age. Four newborn rat cardiac tissues were taken for each experiment. After the tissues were separation, digestion, and plate purification for 48 hours, cardiac microvascular endothelial cells with a quantity of $>1\times10^6$ cells can be obtained. After separation, cells can be passaged 2-3 times in a 1:2 ratio and cultured for 1-2 weeks.

Note:Extracting complete cardiac tissue from 4 rats can obtain cells in a T25 culture flask, and the specific number of rats required may vary depending on the amount of complete cardiac tissue obtained. If the amount of tissue obtained is small, the amount of experimental rats can be appropriately increased to avoid insufficient cell count.

Components of Kit

The components of this kit is shown in the table below

Table.1.Composition and corresponding information of rat cardiac microvascular endothelial cells isolation and culture kit

Name	Size	Appearance	Storage and Expiration Date
Specialized Washing Solution For Rat Cardiac Microvascular Endothelial Cells	3Tests (250 mL) 10Tests (500 mL)	Faint Yellow Clear Liquid	2-8°C, 1 year
Digestive Solution A For Rat Cardiac Microvascular Endothelial Cells	3Tests (15 mL) 10Tests (50 mL)	Colorless Clear Liquid	-5~-20°C, 1 year
Digestive Solution B For Rat Cardiac Microvascular Endothelial Cells	3Tests (3 mL) 10Tests (10 mL)	Colorless Clear Liquid	-5~-20°C, 1 year
Basic Culture Medium For Rat Cardiac Microvascular Endothelial Cells	3Tests (100 mL) 10Tests (250 mL)	Red Clear Liquid	2-8°C, 1 year
Supplement For Rat Cardiac Microvascular Endothelial Cells	3Tests (10 mL) 10Tests (25 mL)	Yellow Clear Liquid	-5~-20°C, 1 year
Specialized Isolation Solution A For Rat Cardiac Microvascular Endothelial Cells	3Tests (10 mL) 10Tests (30 mL)	Faint Yellow Clear Liquid	2-8°C, 1 year
Specialized Isolation Solution B For Rat Cardiac Microvascular Endothelial Cells	3Tests (15 mL) 10Tests (50 mL)	Faint Yellow Clear Liquid	2-8°C, 1 year

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Name	Size	Appearance	Storage and Expiration Date
Planting Solution For Rat Cardiac Microvascular Endothelial Cells	3Tests (3 mL) 10Tests (10 mL)	Colorless Clear Liquid	-5~-20°C, 1 year
70 μm Cell Filter	3Tests (3 pcs) 10Tests (10 pcs)	Orange	Room temperature, 3 years

Note: Please store each component according to the temperature indicated on the label on the reagent tube. After thawing, the digestive fluid should be stored at 4℃ for 30 days. It is recommended to divide the digestive fluid according to the instructions after the first use of the reagent kit, freeze it in a -20℃ refrigerator, and thaw it again before use to avoid repeated freezing and thawing.

Notes

1. Before the formal experiment, it is recommended to use 1-2 normal rats for simulated anatomy to familiarize the operation process and improve the speed of tissue separation.
2. During the entire anatomical sampling, it is recommended to place the culture dish containing the tissue on an ice plate (2-8°C) to maintain low temperature, but be careful not to freeze the tissue and liquid due to low temperature.
3. The culture medium contains nutrients necessary for microbial growth. Please open it in a super clean workbench, divide it according to the required amount, and seal the bottle mouth with a sealing film for immediate use to avoid contamination.
4. All separation solutions in this kit may experience the precipitation of crystal particles at the bottle mouth and turbidity of the solution during storage, which is a normal phenomenon and can be used with no problem.

Operational Procedures

1. Pre-experiment Preparation

(1) Reagent Thawing and Rewarming:

a. **Digestive Solution A For Rat Cardiac Microvascular Endothelial Cells, Digestive Solution B For Rat Cardiac Microvascular Endothelial Cells, Supplement For Rat Cardiac Microvascular Endothelial Cells, Planting Solution For Rat Cardiac Microvascular Endothelial Cells**: Thaw at 4°C and equilibrate to room temperature.

b. **Specialized Washing Solution For Rat Cardiac Microvascular Endothelial Cells, Specialized Isolation Solution A For Rat Cardiac Microvascular Endothelial Cells, Specialized Isolation Solution B For Rat Cardiac Microvascular Endothelial Cells, Basic Culture Medium For Rat Rat Cardiac Microvascular Endothelial Cells**: Equilibrate to room temperature.

(2) Additional Materials Required: Ice plate, surgical instruments (At least 3 ophthalmic scissors, 1 straight tweezers, and 2 curved tweezers are included), 6 cm/10 cm culture dish, Dissection plate (can be replaced by foam plate), assorted 2 mL/15 mL/50 mL centrifuge tubes.

(3) Preparation of **Complete Culture Medium For Rat Cardiac Microvascular Endothelial Cells**: Add 1 mL of **Supplement For Rat Cardiac Microvascular Endothelial Cells** into 10 mL of **Basic Culture Medium For Rat Cardiac Microvascular Endothelial Cells**, mix thoroughly.

(4) Coating of CultureVessels: Add 1 mL **Planting Solution For Rat Cardiac Microvascular 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 disinfection and euthanasia: Euthanize the experimental rat and immerse them in 75% medical alcohol for 5 minutes for disinfection. After sterilization, transfer the animal to a clean bench for subsequent procedures.

(2) Dissection and Tissue Harvesting Steps:

a. Preparation work: Place two clean orifice plates in the clean workbench, and place ophthalmic scissors 1, straight tweezers 1, ophthalmic scissors 2, curved tweezers 2, ophthalmic scissors 3, and curved tweezers 3 from left to right above the dissection plates. Pay attention to placing ophthalmic scissors and tweezers in pairs, with about one-third of the front part suspended. After use, place the scissors and tweezers back in their original positions without touching each other to prevent contamination.

b. Rat fixation: Secure the rat in a supine position within the clean workbench using needles for stabilization during tissue harvesting.

c. Anatomical procedure: Use straight tweezers 1 to lift the skin of the experimental rat, and ophthalmic scissors 1 to cut open the chest skin and the attached flesh membrane tissue below. Use straight tweezers 1 to pull the skin towards the neck and exchange it for another set of ophthalmic scissors 2 and curved tweezers 2. Use curved tweezers 2 to clamp the lower right rib arch of the rat, and use ophthalmic scissors 2 to cut the rib upwards to the clavicle, horizontally cut the diaphragm, and cut along the lower left rib to the clavicle of the left scapula. Cut the sternum handle, flip it upwards, fully open the chest cavity, and expose the cardiac.

d. Sampling procedure: Cut open the blood vessels connected to the cardiac using ophthalmic scissors 2, remove the complete cardiac tissue, and transfer the tissue to a new glass culture dish. Add 10 mL of **Specialized Washing Solution For Rat Cardiac Microvascular Endothelial Cells** to the culture dish in advance. Then, place the entire petri dish on an ice tray/ice box to maintain a low-temperature environment.

3. Tissue Processing and Digestion

(1) Tissue Washing: Use for a new set of ophthalmic scissors 3 and curved forceps 3, rinse the tissue once to remove excess blood stains and cut off the connective tissue above the cardiac (as shown in Figure 1), transfer the tissue to a new glass culture dish, and pre add 10 mL of **Specialized Washing Solution For Rat Cardiac Microvascular Endothelial Cells** to the dish.

Note: Optional steps - Use ophthalmic scissors 3 to cut open the cardiac diagonally, retain the ventricular part. Use microscopic scissors to remove 1/4 of the outer layer of the ventricular tissue, which contains more fibroblasts. Cutting off this layer of tissue can reduce fibroblast contamination and retain the inner tissue of the cardiac (as shown in Figure 2).

(2) Tissue processing: Transfer the tissue to a 1.5 mL EP tube, add **Complete Culture Medium For Rat Cardiac Microvascular Endothelial Cells** until it has just immersed the tissue, cut it into 1 mm^3 pieces using ophthalmic scissors 3, and transfer the tissue to a 15 mL centrifuge tube using a pipette. Add 5 mL of **Specialized Washing Solution For Rat Cardiac Microvascular Endothelial Cells**, repeatedly blow the tissue inside the tube with a 5 mL pipette 5 times, centrifuge at 300 g for 1 minute, discard the supernatant, and retain the tissue precipitate.

(3) Tissue digestion:

a. Add 5 mL of **Digestive Solution A For Rat Cardiac Microvascular Endothelial Cells** into a 15 mL centrifuge tube, blow and mix well; Place the centrifuge tube diagonally into a 37°C water bath shaker at a speed of 150 rpm and digest for 1 hour.

b. After digestion is complete, remove the centrifuge tube and add 1 mL of **Digestive Solution B For Rat Cardiac Microvascular Endothelial Cells.** Place the centrifuge tube diagonally in a 37°C water bath shaker at a speed of 150 rpm and digest again for 30 minutes.

c. During the digestion process, remove a **70 μm Cell Filter** and place it over a 50 mL centrifuge tube. Rinse the cell filter with 2 mL of washing solution.

d. After digestion is complete, remove the centrifuge tube and repeatedly blow it with a pipette 20-30 times until there are no obvious tissue blocks. Add 5 mL of **Specialized Washing Solution For Rat Cardiac Microvascular Endothelial Cells** to the centrifuge tube, gently blow and mix well, filter it through a 70 μm cell filter that has been moistened, and collect the filtrate in a 50 mL centrifuge tube.

4. Cell isolation

(1) Collect the filtrate in a 50 mL centrifuge tube, centrifuge 300 g for 5 minutes; Discard the supernatant and retain the precipitate.

(2) Resuspend the retained precipitate in 5 mL of washing solution, centrifuge again at 300 g for 5 minutes. After centrifugation, discard the supernatant and retain the precipitate. Resuspend the precipitate with 3 mL **Specialized Isolation Solution A For Rat Cardiac Microvascular Endothelial Cells** .

(3) Take a new 15 mL centrifuge tube and add 5 mL of **Specialized Isolation Solution B For Rat Cardiac Microvascular Endothelial Cells** for later use.

(4) Tilt the 15 mL centrifuge tube prepared in step (3) and use a 1 mL pipette to aspirate the cell suspension obtained in step (2). Place the pipette tip against the wall of the 15 mL tube and gently blow the cell suspension, slowly adding it along the tube wall to the top of the **Specialized Isolation Solution B For Rat Cardiac Microvascular Endothelial Cells**, forming a liquid level stratification.

Note: If it is not ensure that form two layers of liquid, a 200 μL pipette can be used to aspirate the resuspended cell suspension and slowly blow out it along the tube wall for about 5-6 shots according to the above operation. After obvious liquid level stratification is seen, use a 1 mL pipette to slowly add the remaining suspension. The liquid level stratification can be observed under light, and a clear boundary line can be seen between the two separation solutions.

(5) Centrifuge 900 g for 15 minutes (set the acceleration to 1 and the decelerate to 0).

(6) After centrifugation, there were obvious microvascular segments between **Specialized Isolation Solution A For Rat Cardiac Microvascular Endothelial Cells** and **Specialized Isolation Solution B For Rat Cardiac Microvascular Endothelial Cells** (as shown in Figure 3, hereinafter referred to as the white membrane layer). First, the top dead cells and fragments were aspirated, and a new pipette tip was used to extend above the white membrane layer. Carefully aspirate the cells from the white membrane layer and transfer them to a new sterile 15 mL centrifuge tube.

(7) Add 5 mL of **Specialized Washing Solution For Rat Cardiac Microvascular Endothelial Cells** to a 15 mL centrifuge tube to resuspend white membrane cells, centrifuge at 300 g for 5 minutes, Discard the supernatant and retain the precipitate.

5. Cell culture and passage

(1) Take out the T25 cell culture flask, discard the **Planting Solution For Rat Cardiac Microvascular Endothelial Cells**, add 5 mL of **Specialized Washing Solution For Rat Cardiac Microvascular Endothelial Cells** (slowly add along the wall when adding the liquid, do not wash the bottom of the bottle), rinse the bottom of the flask, and then discard the washing solution. Put in a 5% CO_2 incubator at 37°C for later use.

(2) Resuspend the cell pellet in a centrifuge tube using 5 mL of **Complete Culture Medium For Rat Cardiac Microvascular Endothelial Cells**, and inoculate it into T25 flask that have been coated. Observe the cells under a microscope, and clearly visible microvascular tissue floats in the culture medium (as shown in Figure 4). Place the cell culture flask in a 37°C, 5% CO_2 constant temperature incubator for static cultivation.

(3) After 48 hours of cell culture, a large number of dead cells, fragments, red blood cells, etc. can be seen above the adherent cells. Do not rinse the bottom of the flask directly, as this may cause the cells to be washed away. Instead, slowly aspirate the supernatant, add 2-3 mL of PBS for rinsing, gently tap the bottom of the bottle to remove debris, aspirate PBS, add fresh **Complete Culture Medium For Rat Cardiac Microvascular Endothelial Cells**, and then change the medium every 2-3 days.

(4) Cell passage

a. When cells reach 80-90%confluency, passage can begin. Discard the original supernatant, add 2-3 mL of PBS to rinse the cells, and discard the PBS.

b. Add 1 mL of Accutase digestion solution (do not use trypsin as it can cause cell death), gently shake until the digestion solution infiltrates all cells in the flask, and digest in a 37°C incubator for 5 minutes. After most of the cells have contracted and become round, add 3-5 mL of **Complete Culture Medium For Rat Cardiac Microvascular Endothelial Cells** to terminate digestion. Gently blow the bottom and collect the cell suspension in a 15 mL centrifuge tube.

c. After centrifugation for 5 minutes at 300 g, discard the supernatant and resuspend the cell pellet in 5 mL of **Complete Culture Medium For Rat Cardiac Microvascular Endothelial Cells.**

d. Gently blow and disperse the cells, inoculate them in separate bottles according to the passage ratio or experimental requirements, and place them in a cell culture incubator at 37°C, 5% CO_2, and saturated humidity for static cultivation.

Troubleshooting

Table.2.Common problems, causes, and solutions of Rat Cardiac Microvascular Endothelial Cells

Problem	Possible Cause	Solution
Low cell quantity.	Inadequate/excessive digestion.	Check the storage conditions of digestivesolution to ensure that they have not been stored at 4°C for more than 30 days, and strictly control the two-step digestion time.
	Excessive tissue sampling leads to inadequate digestion and insufficient freshness of the tissue.	Ensure that the sedimentation amount of the tissue does not exceed 1/3 of the digestive solution, cut the tissue as much as possible, and take tissue from rat that have been euthanized for a short time.

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Problem	Possible Cause	Solution
Cell proliferation is slow.	Improper preservation and preparation of culture medium.	Ensure that the proportion of the complete culture medium is correct, the additives are not repeatedly frozen or thawed, and the preparation time does not exceed three months.
Cell proliferation is slow.	Older rat are prone to slow cell proliferation and reduced cell passage times.	Ensure that the age of the rat is at least 14 days and the maximum age does not exceed 30 days.
	Improper passage ratio and over passage may result in slower cell proliferation as the number of passages increases.	Ensure that the number of cell passages is 1:2, not exceeding 2-3 times, converted according to the bottom area of the vessel, to ensure that the initial number of cells inoculated is sufficient.
Low cell purity.	Improper layered suction operation resulted in contamination when the tip came into contact with cells in other layers.	When using a separation solution for separation, avoid the upper layer of tissue touching the lower layer of sediment and try to completely absorb the upper layer of tissue in the separation solution.
	Improper sampling resulted in contamination of the endothelium of large blood vessels.	Take 3/4 of the cardiac tissue near the apex of the heart.
Significant loss of cells.	After being attached to the wall, cells may detach due to severe treatments such as washing or vigorous tapping.	According to step (2) of process 5, after 2-3 days of cell adhesion, do not rinse the bottom of the flask with PBS, culture medium, etc. when changing the medium, and gently tap the bottom of the flask.

Anatomy Images for Reference

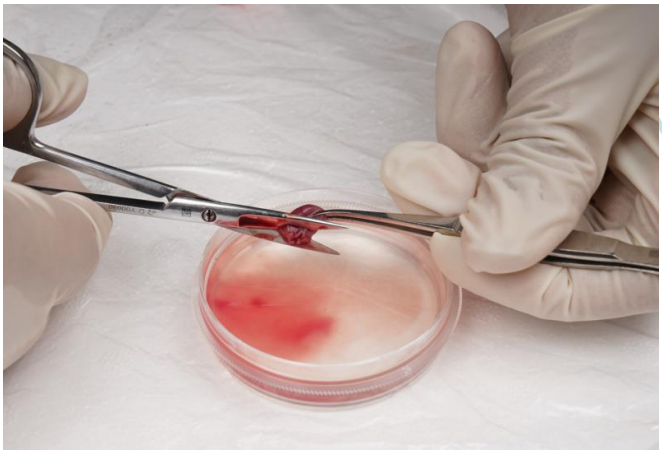

Figure 1. Cutting open the blood vessels above the cardiac tissue

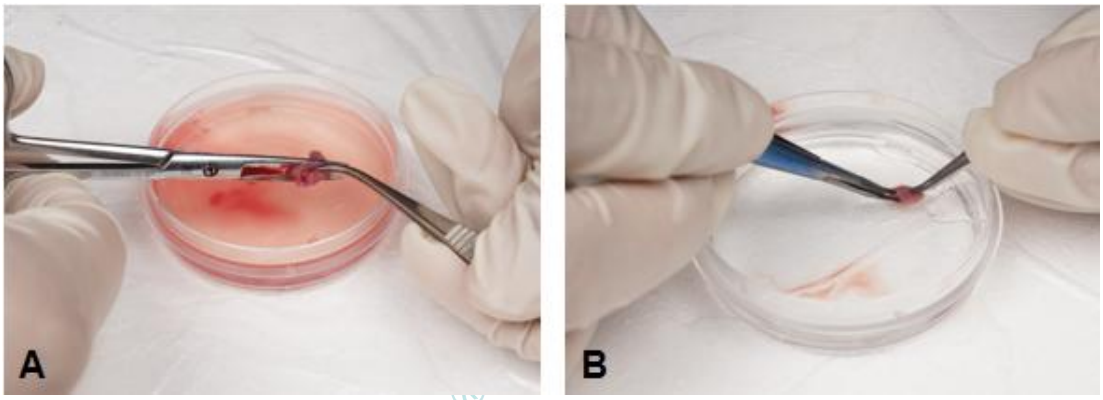

Figure 2. Sampling of tissues from the inner part of the ventricle
A: Cut open the cardiac diagonally, leaving the ventricular part; B: Fix the tissue with curved tweezers and use micro scissors to remove approximately 1/4 of the extra ventricular area

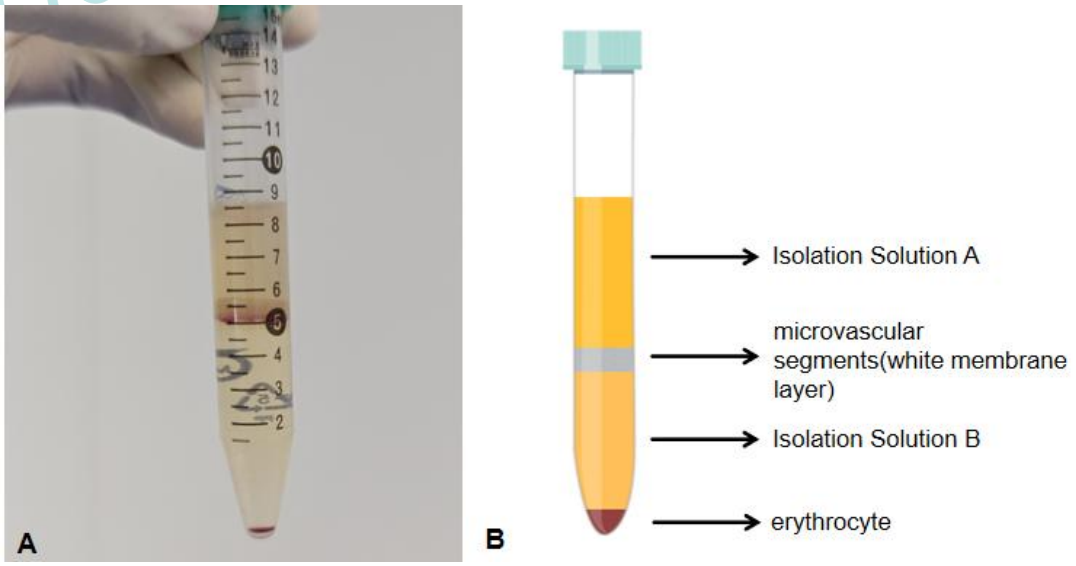

Figure 3. Purification of cells by separation solution
A: Real image of cells purified by separation solution; B: Schematic diagram of cells purified by separation solution.

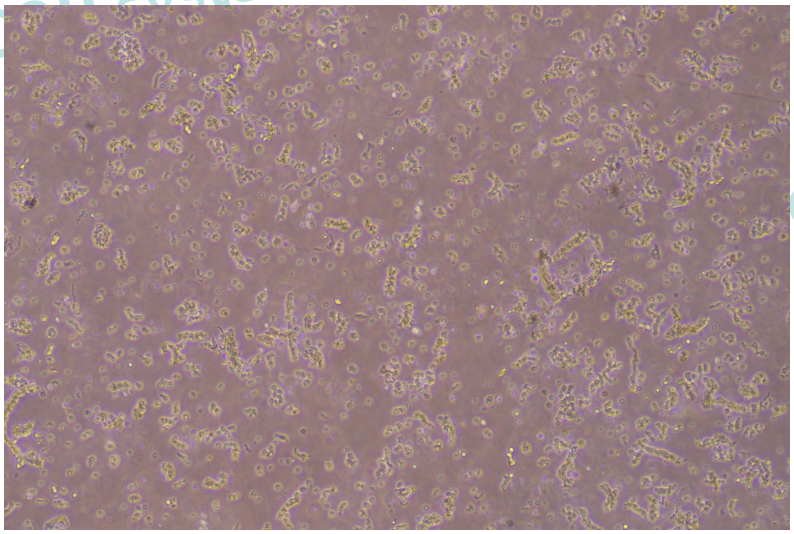

Figure 4. The cell suspension immediately after inoculation shows distinct microvascular tissue Segments (For reference only, the number and size of microvascular segments may vary depending on experimental conditions)