Rat Cardiomyocyte Isolation and Culture Kit

Cat.No.: P-CA-621

Size: 3Tests / 10Tests

Background

This kit is specially developed for extracting primary rat cardiomyocyte. It uses low-temperature digestion and differential adhesion methods to isolate and purify rat cardiomyocytes. After laboratory verification, it can isolate more than 1×10^6 target cells in each experiment (1 Test). Immunofluorescence identification (α-Sarcomeric actin) results show that the cell purity is above 90%.

Scope of Application

This product is suitable for extracting cardiomyocytes from different strains of rats such as Wistar and SD at 1-2 days of age. Five newborn rat heart tissues were taken for each experiment. After digestion, separation, and plate purification for 48 hours, myocardial cells with a quantity of >1 × 10^6 cells can be obtained. Myocardial cells belong to terminally differentiated cells and do not proliferate. After separation, the cells can be cultured for about 30 days.

Note:Extracting complete heart tissue from 5 rats can obtain cells in a T25 culture flask, and the specific number of rats required may vary depending on the amount of complete heart 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 cardiomyocytes isolation and culture kit

Name	Size	Appearance	Storage and Expiration Date
Special Washing Solution For Rat Cardiomyocytes	3Tests (250 mL) 10Tests (500 mL)	Faint Yellow Clear Liquid	2-8°C,1 year
Special Digestive Solution For Rat Cardiomyocytes	3Tests (15 mL) 10Tests (50 mL)	Colorless Clear Liquid	-5~-20°C,1 year
Basic Culture Medium For Rat Cardiomyocytes	3Tests (100 mL) 10Tests (250 mL)	Red Clear Liquid	2-8°C,1 year
Screening Solution For Rat Cardiomyocytes	3Tests (0.6 mL) 10Tests (2 mL)	Colorless ClearLiquid	2-8°C,1 year
Supplement For Rat Cardiomyocytes	3Tests (10 mL) 10Tests (25 mL)	Yellow Clear Liquid	-5~-20°C,1 year
Planting Solution For Rat Cardiomyocytes	3Tests (3 mL) 10Tests (10 mL)	Colorless Clear Liquid	-5~-20℃, 1 year
100 μm Cell Filter	3Tests (3 pcs) 10Tests (10 pcs)	N/A	Room temperature, 3 years
70 μm Cell Filter	3Tests (3 pcs) 10Tests (10 pcs)	N/A	Room temperature, 3 yearss

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. This product is only used for scientific research or further research, not for diagnosis and treatment.
2. 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.
3. The isolation process of myocardial cells requires high cell activity. Please euthanize the experimental rat and immediately proceed with the experimental operation.
4. **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, so as not to damage the tissue and affect the extraction efficiency.**
5. The culture medium contains nutrients necessary for microbial growth. Please open it in a super clean bench, divide it according to the required amount, and seal the bottle mouth with a sealing film for immediate use to avoid contamination.

Operational Procedures

1. Pre-experiment Preparation

(1) Reagent Thawing and Rewarming: **Special Digestive Solution For Rat Cardiomyocytes, Supplement For Rat Cardiomyocytes, Planting Solution For Rat Cardiomyocytes**: Thaw at 4℃ and equilibrate to room temperature. **Special Washing Solution For Rat Cardiomyocytes, Basic Culture Medium For Rat Cardiomyocytes, Planting Solution For Rat Cardiomyocytes:** Equilibrate to room temperature.

(2) Additional Materials Required: EP tube, 0.25% Trypsin digestion solution, Dissection plate (can be replaced by foam plate), Ice plate, PBS, surgical instruments (At least 3 ophthalmic scissors, 1 straight tweezers, and 2 curved tweezers are included), Tissue processing dish (glass dish, 6 cm/10 cm is acceptable), T25 culture flask, assorted 2 mL/15 mL/50 mL centrifuge tubes.

(3) Preparation of culture medium

a. **Complete Culture Medium For Rat Cardiomyocytes**: Add 1 mL of **Supplement For Rat Cardiomyocytes** into 10 mL of **Basic Culture Medium For Rat Cardiomyocytes**, mix thoroughly.

b. **Screening Culture Medium For Rat Cardiomyocytes**: Add 200 μL of **Screening Solution For Rat Cardiomyocytes** into 5 mL of **Complete Culture Medium For Rat Cardiomyocytes**, mix thoroughly.

(4) Coating of CultureVessels: Add 1 mL **Planting Solution For Rat Cardiomyocytes** 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) 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 bench, 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 orifice 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 prone position within the clean bench using needles for stabilization during tissue harvesting.

c. Anatomy: Use straight tweezers 1 to fix and clamp the skin on the upper abdomen of a rat. Use ophthalmic scissors 1 to open from the upper abdomen and cut open the skin from the opening to both sides of the clavicles. Use straight tweezers 1 to lift up the cut open skin, and use ophthalmic scissors 1 to cut off the attached fascia tissue, fully exposing the sternum. Clamp the ribs with curved forceps 2, use ophthalmic scissors 2 to sequentially cut open the peritoneum and thoracic septum, and cut open the sternum and ribs along the right side of the xiphoid cartilage, exposing the heart tissue.

d. Sampling: Use a curved forceps to clamp the upper part of the atrium, cut open the blood vessels connected to the heart using ophthalmic scissors, remove the complete heart tissue, and transfer the tissue to a new glass culture dish (as shown in Figure 1). Add 10 mL of **Special Washing Solution For Rat Cardiomyocytes** to the culture dish in advance.

3. Tissue Processing and Digestion

(1) Organizational cleaning: Use a new set of ophthalmic scissors and curved forceps to rinse the tissue once, remove excess blood and connective tissue, transfer the heart tissue to a new glass culture dish, and add 10 mL of **Special Washing Solution For Rat Cardiomyocytes** to the dish in advance.

(2) Organizational processing: Use ophthalmic scissors 3 to horizontally cut the tissue at a distance of 1/2 from the apex of the heart (as shown in Figure 2. A), retaining only the apex of the heart and discarding excess tissue. Transfer the retained tissue to a 1.5 mL EP tube, add a small amount of **Complete Culture Medium For Rat Cardiomyocytes** until it just covers the tissue, use ophthalmic scissors 3 to cut the tissue inside the tube into 1 mm^3 fragments (as shown in Figure 2. B), and transfer the tissue to a 15 mL centrifuge tube using a pipette. Add 5 mL of **Special Washing Solution For Rat Cardiomyocytes**, repeatedly blow the tissue in the tube with a 5 mL pipette 5 times, centrifuge at 500 rpm for 1 minute, discard the supernatant, and retain the tissue precipitate.

(3) Tissue digestion: Add 5 mL of **Special Digestive Solution For Rat Cardiomyocytes** into a 15 mL centrifuge tube, blow and mix well. Place the centrifuge tube flat in a 4°C refrigerator for 2 hours. After digestion is complete, remove the centrifuge tube and place it in a 37°C water bath shaker at 150 rpm for 15 minutes.

(4) Place a **70 μm Cell Filter** and **100 μm Cell Filter** on a new 50 mL centrifuge tube. Pre-wash the filter with 1-2 mL washing solution.

(5) After digestion is complete, gently blow the tissue in the tube 5 times with a 5 mL pipette, add 5 mL of **Complete Culture Medium For Rat Cardiomyocytes** to the tube to terminate digestion, and repeatedly blow with a pipette 20-30 times until there are no obvious tissue blocks. Filter through a 100 μm cell filter and a 70 μm cell filter in sequence, and collect the filtrate in a 50 mL centrifuge tube.

Note: If the suspension filtration is slow or unable to be filtered during this step, it may be due to the tight fit between the cell filter and the centrifuge tube mouth. At this time, you can try tilting the cell filte slightly against the tube rim to improve this phenomenon.

(6) Cell separation: Centrifuge the 50 mL centrifuge tube from the previous step at 1000 rpm for 5 minutes; Discard the supernatant and retain the sediment. After resuspending the precipitate in 5 mL of washing solution (gently operate to avoid damaging the cells and avoiding direct blow of the precipitate, use a pipette to disperse, the same below), centrifuge at 1000 rpm for 5 minutes, discard the supernatant, and retain the precipitate

4. Cell culture, morphological assessment, and passage

(1) Cell seeding: Take out the pre-coated T25 cell culture flask and aspirate the **Planting Solution For Rat Cardiomyocytes**. Slowly add **Specialized Washing Solution For Rat Rat Cardiomyocytes** to wash the flask twice, 5 mL each time, along the inner wall of the flask to avoid disrupting the coated surface. Aspirate the washing solution after gentle rinsing.Put in a 5% CO_2 incubator at 37°C for later use.

(2) Resuspend the precipitate from the previous step in 5 mL of **Complete Culture Medium For Rat Cardiomyocytes**, inoculate it into a culture dish, and incubate it in a constant temperature incubator at 37°C and 5% CO_2; After 45 minutes of cultivation, take out the culture dish, gently tap the bottom of the dish to remove the non adherent cells, resuspend them in the culture medium, and transfer the cell culture supernatant from the dish to a new 15 mL centrifuge tube. Centrifuge at 1000 rpm for 5 minutes, discard the supernatant, and retain the precipitate.

(3) Resuspend the precipitate obtained in the previous step in 5 mL of **Screening Culture Medium For Rat Cardiomyocytes** and inoculate it into T25 flask for screening and culture. After 48 hours, replace it with **Complete Culture Medium For Rat Cardiomyocytes** and change the medium every 2-3 days thereafter.

(4) Morphological assessment of myocardial cells: After 24 hours of culture, myocardial cells are basically adhered to the bottom, and individual cells spontaneously beat, but the beat frequency and rhythm are different; After 48 hours, the myocardial cells extended pseudopodia, and initial connections were observed between cells. Most cells produced pulsations, which tended to synchronize; After 72 hours, the cells are connected into a network with increased pulsation, and the shape changes from flat, spindle shaped, triangular to irregular star shaped. Multiple or single nuclei may appear, but single nuclei are more common. The nucleus is the round and the nucleolus is clearer; After 96 hours, most cells will produce pseudopodia, which are relatively clear, with a more pronounced cell pulsation frequency and an average increase in frequency. The vast majority of cells will become irregular star shaped. After 120 hours, cells form cell clusters, which exhibit synchronous pulsations. After 7-10 days, digestion can begin and the cells gradually mature. From 40 to 45 days, cell degeneration and death gradually occur.

(5) Cell digestion: Once the cell confluence reaches 80-90%, transfer and digest the cells. Discard the original supernatant and add 2-3 mL of PBS to rinse the cells. Discard the PBS and add 1 mL of 0.25% trypsin digestion solution. Gently shake until all cells are infiltrated into the flask. Discard the trypsin and digest in a 37°C incubator for 1-3 minutes. After most of the cells have contracted and become round, add 3-5 mL of complete culture medium to terminate digestion. Gently blow and disperse the cells, inoculate them into pre packaged experimental vessels, 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 cardiomyocytes

Problem	Possible Cause	Solution
After reheating and digestion, the tissue cannot be blown away, and some tissues still entangle and stick together.	Excessive material quantity and insufficient tissue cutting and processing.	The volume of digestive fluid is greater than or equal to three times that of the tissue block.Ensure to cut the tissue into pieces.
	Digest at 4°C time is relatively short	The time for low-temperature digestion can be appropriately extended, and the digestion time should be controlled within 2-4 hours.
Low cell purity/low yield.	Improper sampling resulted in the use of tissues containing a significant amount of fibroblasts.	Take tissue near the apex of the heart, which has a relatively low content of myocardial fibroblasts.
	The time for differential adhesion cannot be controlled properly. If the differential time is too short, it can cause contamination of fibroblasts, while if it is too long, it can lead to a lower cell yield.(procedure4-step (2))	Try adjusting the time for differential adhesion , increasing/decreasing it by about 10 minutes depending on the specific situation.
During the differential adhesion process, neither culture dish had cell adhesion and the cells floated.	Overdigestion leads to cell death	Control the re-warming digestion time to no more than 15 minutes, and immediately use sufficient complete culture medium to terminate digestion after digestion is complete.
Myocardial cells float and die, but myocardial fibroblasts adhere to the bottom normally.	The sampling time is too long after the death of the rats and the digestion time is too long.	Immediately conduct the experiment after euthanizing the mice, and control the thawing digestion time within 15 minutes.
	Excessive suspension and blowing of cell precipitation resulted in cell death.	Control the frequency and intensity of blowing. (procedure3-step (6), procedure4-step (1))
Myocardial cell clustering	Incomplete digestion, still with cell clusters.	Ensure correct digestion conditions.

Anatomy Images for Reference

Figure 1. Fresh cardiac tissue

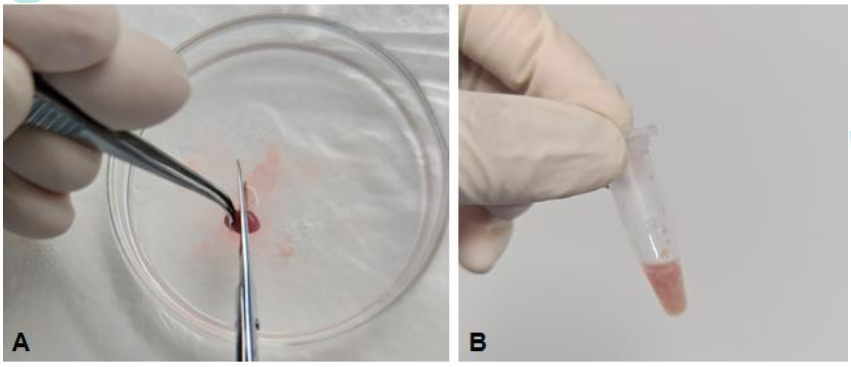

Figure 2. Cardiac tissue processing
A: Use scissors to cut the heart near the apex; B: Reference for the state of heart tissue after being cut into pieces in an EP tube.

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