# Rat Cardiac Fibroblast Isolation and Culture Kit

**Cat.No.: P-CA-622**

**Size: 3Tests / 10Tests**

## Background

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

## Scope of Application

This product is suitable for extracting cardiac fibroblasts from different strains of rat such as Wistar and SD at 1-2 days of age. Five newborn rats heart tissues were taken for each experiment. After digestion, separation, and plate purification, cardiac fibroblasts with a quantity of >1 × $10^6$ cells can be obtained, the cells can be passaged for at least 3 generations in a 1:2 ratio,it can be cultured for about two weeks.

**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 cardiac fibroblasts isolation and culture kit

| Name | Size | Appearance | Storage and Expiration Date |
|---|---|---|---|
| Special Washing Solution For Rat Cardiac Fibroblasts | 3Tests (250 mL)<br>10Tests (500 mL) | Faint Yellow Clear Liquid | 2-8°C, 1 year |
| Specialized Digestive Solution For Rat Cardiac Fibroblasts | 3Tests (50 mL)<br>10Tests (150 mL) | Colorless ClearLiquid | -5~-20°C, 1 year |
| Digestive Termination Solution For Rat Cardiac Fibroblasts | 3Tests (10 mL)<br>10Tests (30 mL) | Yellow Clear Liquid | -5~-20°C, 1 year |
| Basic Culture Medium For Rat Cardiac Fibroblasts | 3Tests (100 mL)<br>10Tests (250 mL) | Red Clear Liquid | 2-8°C, 1 year |
| Supplement For Rat Cardiac Fibroblasts | 3Tests (10 mL)<br>10Tests (25 mL) | Yellow Clear Liquid | -5~-20°C, 1 year |
| 100 μm Cell Filter | 3Tests (3 pcs)<br>10Tests (10 pcs) | N/A | Room temperature, 3 years |
| 70 μm Cell Filter | 3Tests (3 pcs)<br>10Tests (10 pcs) | N/A | 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. 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. 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.
4. 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 Cardiac Fibroblasts, Supplement For Rat Cardiac Fibroblasts, Digestive Termination Solution For Rat Cardiac Fibroblasts**: Thaw at 4°C and equilibrate to room temperature. **Special Washing Solution For Rat Cardiac Fibroblasts,Basic Culture Medium For Rat Cardiac Fibroblasts**: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. If need to expand the cultivation, please prepare own complete culture and trypsin.

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

### 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 Cardiac Fibroblasts** 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 Cardiac Fibroblasts** to the dish in advance.

(2) Organizational processing: Use ophthalmic scissors 3 to horizontally cut the tissue at a distance of 3/4 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 Cardiac Fibroblasts** 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 Cardiac Fibroblasts**, 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:

a. Add 5 mL of **Special Digestive Solution For Rat Cardiac Fibroblasts** into a 15 mL centrifuge tube, blow and mix well. Place the centrifuge tube in a 37°C water bath shaker at 150 rpm for 8 minutes.

b. Remove the centrifuge tube and use a 5 mL pipette to repeatedly blow 15 mL of tissue into the centrifuge tube 20-30 times. Add 5 mL **Special Washing Solution For Rat Cardiac Fibroblasts** of to the centrifuge tube, centrifuge at 300 rpm for 3 minutes, collect the supernatant in a 50 mL centrifuge tube, add 1 mL of **Digestive Termination Solution For Rat Cardiac Fibroblasts** to the 50 mL tube to terminate digestion, and prepare the tissue precipitate in the 15 mL centrifuge tube for later use.

c. Repeat steps a and b of tissue digestion twice for the remaining tissue precipitate in a 15 mL centrifuge tube, collect all in a 50 mL centrifuge tube, and blow until no obvious tissue fragments is present after digestion.

(4) Cell collection:

a. 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.

b. Filter the single-cell suspension collected from the previous digestion step through a **100 μm Cell Filter** and a **70 μm Cell Filter** in sequence..

**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.**

c. Centrifuge the 50 mL centrifuge tube from the previous step at 1200 rpm for 5 minutes; Discard the supernatant and retain the sediment. After resuspending the precipitate in 10 mL of washing solution, centrifuge at 1200 rpm for 5 minutes, discard the supernatant, and retain the precipitate.

## 4. Cell culture and passage

(1) Cell purification

a. Take out theT25 cell culture flask .Resuspend the precipitate from the previous step in 5 mL of **Complete Culture Medium For Rat Cardiac Fibroblasts**, and incubate it in a constant temperature

incubator at 37°C and 5% $CO_2$ for 1 hour.

b. After cultivation, most of the adherent cells are cardiac fibroblasts, and the supernatant contains the remaining non adherent miscellaneous cells. Gently tap the bottom of the dish to release the cells adhered to the bottom but not adherent into the supernatant, and transfer the supernatant to another new 6 cm culture dish for observation and use, in order to investigate possible problems in the future.

c. Cultivate with 5 mL of fresh **Complete Culture Medium For Rat Cardiac Fibroblasts**, continue to change the medium after 24 hours, and then change the medium every 2-3 days thereafter.

(2) Cell passaging

a. Passage should be initiated when cells reach 80-90% confluency. Aspirate the old medium and rinse cells with 2-3 mL PBS to remove residual serum.

b. Add 1 mL of 0.25% trypsin solution to the flask. Tilt the flask gently to ensure even coverage of the cell monolayer. Aspirate excess trypsin. Place the flask in a 37°C incubator for 1-3 minutes.

c. Monitor under an inverted microscope until > 80% of cells round up and detach. Add 3-5 mL **Complete Medium Rat Cardiac Fibroblasts** to neutralize trypsin activity. Pipette gently to resuspend cells into a single-cell suspension. Transfer the cell suspension to new culture flasks at the desired split ratio. Ensure even distribution by swirling the flask. Incubate the flasks at 37°C in a humidified atmosphere with 5% $CO_2$ and saturated humidity.

## Troubleshooting

Table.2.Common problems, causes, and solutions of rat cardiomyocytes

| Problem | Possible Cause | Solution |
|---|---|---|
| The cell digestion effect is unsatisfactory | Digesting the tissue in a 37°C incubator resulted in insufficient heating of the digestion solution in the centrifuge tube. | Shake the tube under water bath conditions. If the water bath does not have oscillation function, please ensure to take out the centrifuge tube and shake it 1-2 times during each digestion process. |
| | When centrifuging the supernatant during three digestion intervals, the tissue was not blown, but centrifuged directly. | After each digestion, gently blow the tissue about 10 times to make it looser and easier to digest. |
| | Repeated freezing and thawing of digestive solution, decreased enzyme activity due to exceeding storage time, improper storage conditions, etc. | Please use digestive solution within 2 months after thawing at 4℃ and store them at 4°C. Do not repeatedly freeze and thaw. |
| Low cell purity/low yield | The time for differential adhesion cannot be controlled properly. | Observe whether the dish observed in steps (1) of process 4 contains target cells and other cells, and adjust the differential adhesion time according to the actual situation. |
| | Failure to add Termination Solution in a timely when taking the supernatant after each digestion step resulted in death due to excessive digestion. | Control digestion time and timely add termination solution. |

Continued

| Problem | Possible Cause | Solution |
|---|---|---|
| Low cell purity/low yield | The flask contains some myocardial cells. | Use trypsin to digest the cells at the bottom of the dish, and perform a second differential adhesion to remove myocardial cells from the digested cells. |
| Slow cell growth | Rats that are too old are prone to slow cell proliferation and reduced cell passage times. | Using newborn rats as experimental subjects, it is not recommended to exceed a maximum age of 3 days. |
| | Improper passage ratio and excessive passage times may result in slower cell proliferation as the number of passages increases. | Ensure that the number of cell passages is within 5 generations, and convert according to the bottom area of the vessel to ensure the initial number of cells inoculated. |

## Anatomy Images for Reference

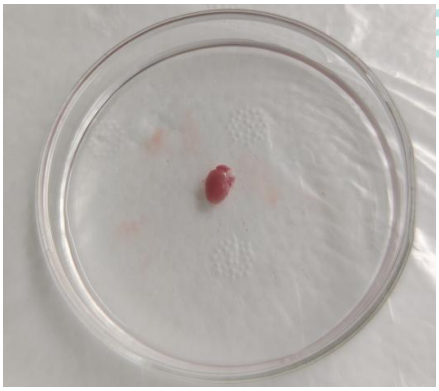


**Figure 1. Fresh cardiac tissue**

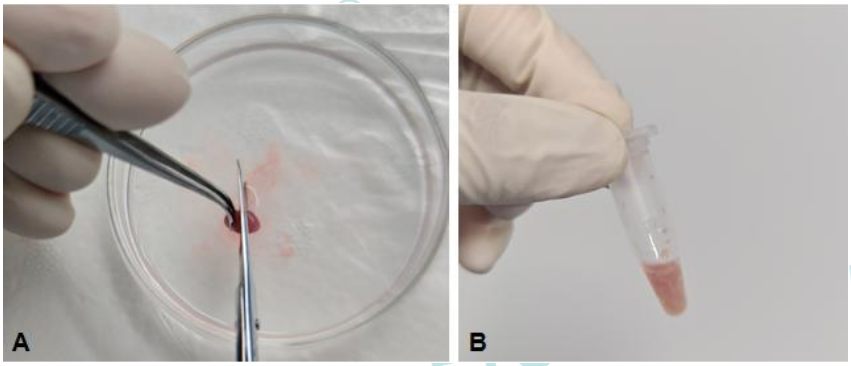



**Figure 2. Cardiac tissue processing**
**A: Use scissors to cut the heart near the apex; B: Reference for the state of heart tissue after being cut into pieces in an EP tube.**