Rat Bone Marrow-Derived Endothelial Progenitor Cell Isolation and Culture Kit

Cat. No.: P-CA-616

Size: 3Tests / 10Tests

Background

The Rat Bone Marrow-Derived Endothelial Progenitor Cell Isolation and Culture Kit is specifically developed for the extraction of primary rat bone marrow-derived endothelial progenitor cells. Verified through standardized procedures, each 1 Test of this kit enables the acquisition of one flask of cells (T-25 culture flask), with a cell count exceeding 1×10^6 cells. When subcultured at a 1:2 ratio, the cells can undergo 1-2 passages. Through immunofluorescence analysis, the cell purity (CD34-positive rate) has been confirmed to exceed 90%.

Scope of Application

This product is suitable for extracting Rat Bone Marrow-Derived Endothelial Progenitor Cells from 20-30-day-old rats of various strains, such as Wistar and SD. After processes of tissue isolation, and 10-14 days of plating, a yield of $>1\times10^6$ cells can be obtained.

Note: The intact tibia and femur tissue extracted from 1 rat, typically yield enough cells for one T-25 flask. The exact number of rats required may vary depending on the size and quantity of tibia and femur tissue harvested during this procedure. If the amount of tissue obtained is insufficient, additional experimental rats may be needed to prevent cell quantity deficiency.

Kit Components

Name	Size	Appearance	Storage and Expiration Date
Specialized Washing Solution For Rat Bone Marrow-Derived Endothelial Progenitor Cells	3Tests (250 mL) 10Tests (500 mL×2)	Pale Yellow Clear Liquid	2-8°C, 1 year
Specialized Lysis Solution For Rat Bone Marrow-Derived Endothelial Progenitor Cells	3Tests (15 mL) 10Tests (50 mL)	Colorless Clear Liquid	2-8°C, 1 year
Basic Culture Medium For Rat Bone Marrow-Derived Endothelial Progenitor Cells	3Tests (100 mL) 10Tests (300 mL)	Red Clear Liquid	2-8°C, 1 year
Supplement For Rat Bone Marrow-Derived Endothelial Progenitor Cells	3Tests (10 mL) 10Tests (30 mL)	Yellow Clear Liquid	-5~-20°C, 1 year
70 μm Cell Filter	3Tests (3 pcs) 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℃ (such as Supplement for Rat Bone Marrow-Derived Endothelial Progenitor Cells) can be preserved at 4℃ for 30 days after thawing. For long-term storage, aliquot them into single-use portions and store at -20℃. 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 yourself with operational procedures and improve tissue isolation

efficiency.

3. Reagent preparation or aliquoting must strictly adhere to aseptic techniques. 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 Preparations

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, 3 pairs of straight forceps, 3 pairs of curved forceps), 2 mL syringe, 6 cm/10 cm culture dishes, T25 culture flasks, dissection board (foam board can substitute) and multiple 2 mL/15 mL/50 mL centrifuge tubes.
2) Reagent Thawing and Rewarming:
   ① Supplement For Rat Bone Marrow-Derived Endothelial Progenitor Cells: Thaw at 4°C and equilibrate to room temperature.
   ② Specialized Washing Solution For Rat Bone Marrow-Derived Endothelial Progenitor Cells & Basic Culture Medium For Rat Bone Marrow-Derived Endothelial Progenitor Cells & Specialized Lysis Solution For Rat Bone Marrow-Derived Endothelial Progenitor Cells: Equilibrate to room temperature.
3) Preparation of Complete Culture Medium: Add 10 mL of Supplement For Rat Bone Marrow-Derived Endothelial Progenitor Cells into 100 mL of Basic Culture Medium For Rat Bone Marrow-Derived Endothelial Progenitor 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.

2. Dissection Procedures

1) Animal Sterilization and Euthanasia: Perform euthanasia via pentobarbital sodium overdose injection or cervical dislocation, then immerse the carcass 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 in order of use from left to right on two sterilized EP tube racks (Ophthalmic Scissors 1 and Straight Forceps 1, Ophthalmic Scissors 2 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-contact.

   ② Rat Fixation: Secure the rat in a supine position within the clean bench using needles, preparing for tissue harvesting.
   ③ Tissue Harvesting Procedure:
      a. Using Straight Forceps 1 to grasp the instep skin of the hind leg, cut the skin bilaterally from bottom to top with Ophthalmic Scissors 1, and cut up to the abdomen.

      Note: Ensure that the entire leg is exposed, and caput femoris as well as the ankle bone at the heel is not

covered with fur.

b. Hold the ankle bone with Curved Forceps 2 in the left hand, cut through the ankle bone and metatarsal bone with Ophthalmic Scissors 2 in the right hand. Lift the ankle bone with Curved Forceps 2, cut the muscle and skin on the back of the leg to the joint at the root of the thigh. Then, cut through the joint to obtain the intact femur and tibia. Place them into the petri dish and add 10 mL of Specialized Washing Solution For Rat Bone Marrow-Derived Endothelial Progenitor Cells. (Figure 1).

Note: Pay attention to keep hair pulled away from the anatomical area. Before sampling, observe the position of tibia and femur to avoid cutting off the tibia and femur. If cut off, bone marrow will be exposed and bone marrow cavity will come into contact with muscle. The tissue should be discarded to prevent contamination.

3. Tissue Processing and Digestion

1) Tissue Processing

① Put Straight Forceps 3, Curved Forceps 3 and Ophthalmic Scissors 3 on the EP tube rack within the clean bench, ensuring the distal third of each tool suspended.

② Tissue dissection with this set of new scissors and forceps. Rinse the tissues once and place them into a new culture dish containing 10 mL of Specialized Washing Solution For Rat Bone Marrow-Derived Endothelial Progenitor Cells.

③ Bone Tissue Dissection:

a. Use Straight Forceps 3 to fix the tissue, tear and loosen the muscle tissue by Curved Forceps 3. Remove large pieces of muscle tissue and don't need to clean them up very well (Figure 2). Place the bones into a new culture dish containing 10 mL of Specialized Washing Solution For Rat Bone Marrow-Derived Endothelial Progenitor Cells to rinse them back and forth.

b. Use Straight Forceps 3 and Curved Forceps 3 to grasp the bones above and below the knee joint , break apart forcefully against direction of joint movement (Figure 3). Be careful not to break the bones, and separate the intact femur and tibia (Figure 4). Place the tibia and femur into a new culture dish and add 10 mL of Specialized Washing Solution For Rat Bone Marrow-Derived Endothelial Progenitor Cells.

c. Use Straight Forceps 3 and Curved Forceps 3 to remove the residual muscle tissue on the surface of femur and tibia (Figure 5), ensuring the bones remain intact, and leave the pure femur and tibia (Figure 6). Transfer the bones into a new culture dish, add 10 mL of Specialized Washing Solution For Rat Bone Marrow-Derived Endothelial Progenitor Cells. Set aside for later use.

d. Pick up the bones one by one with Curved Forceps 3, and cut off both ends of the bones with Ophthalmic Scissors 3 to expose the bone marrow (Figure 7), then place them in a dry and sterile culture dish.

e. Prepare a new culture dish and add 10 mL of Specialized Washing Solution For Rat Bone Marrow-Derived Endothelial Progenitor Cells. Pick up one bone with Curved Forceps 3 in the left hand, and use a 2 mL syringe in your right hand to aspirate the Specialized Washing Solution For Rat Bone Marrow-Derived Endothelial Progenitor Cells from the culture dish (Figure 8). Insert the needle into the thicker end of the bone and perform

above the culture dish, taking care not to let the bone fall into it. Stir the needle and rinse the bone marrow (Figure 9), continuing the process until the bone turns white and translucent (Figure 10). Perform the same operation on the other bones. Collect the bone marrow fluid in the culture dish (Figure 11) and gently aspirate about 15 times with a 5 mL pipette or a Pasteur pipette.

2) Cell Isolation

① Place a 70 μm Cell Filter on a new 50 mL centrifuge tube. Pre-wash the filter with 1 mL of washing solution.

Use a 5 mL pipette or a Pasteur pipette to carefully aspirate the bone marrow suspension from the previous step and filter it through a 70 μm cell filter. After filtering, change a clean pipette tip to slowly add 2 mL of Specialized Washing Solution For Rat Bone Marrow-Derived Endothelial Progenitor Cells into the filter to collect the bone marrow cells on the filter. Collect the filtrate in a 15 mL centrifuge tube.

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

② Take this filtrate-containing 15 mL centrifuge tube and centrifuge at 1500rpm for 5min. Discard the supernatant and retain the cell pellet. Add 5 mL of Specialized Lysis Solution for Mouse Bone Marrow-Derived Endothelial Progenitor Cells into a 15 mL centrifuge tube to resuspend the precipitate. Place the tube on a centrifuge rack and incubate for 3 minutes. Then add 5 mL of Specialized Washing Solution For Mouse Bone Marrow-Derived Endothelial Progenitor Cells. Transfer the cell suspension to a fresh 15 mL centrifuge tube and centrifuge at 1500 rpm for 5 minutes. Carefully discard the supernatant while retaining the pellet.

4. Cell Culture and Subculture

1) Cell Seeding: Resuspend the cell pellet in the centrifuge tube with 10 mL of Complete Culture Medium For Rat Bone Marrow-Derived Endothelial Progenitor Cells, inoculate the cell suspension into a 6 cm cell culture dish. The cells were cultured in a incubator at 37°C, 5% CO_2 for 18 hours. Collect the culture medium from the dish and inoculate non-adherent cells within it into a T25 cell culture flask, add 5 mL of fresh complete culture medium, gently blow and mix well.

2) Medium Renewal: Perform the first medium change at 18 hours. Subsquent centrifugation and medium replacements should be performed every 2-3 days. After inoculation for about 10-14 days, the cell confluence will reach 80-90%.

3) Cell Subculture: When subcultured at a 1:2 ratio, this cell can undergo 1-2 passages and is not reccomanded for multiple passages. 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 1mL of 0.25% trypsin digestive solution to the T25 flask, gently rotate the flask until the digestive 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 round, then add 5mL of Complete Medium For Rat Bone Marrow-Derived Endothelial Progenitor Cells to terminate the digestion, gently resuspend and disperse the cells, and inoculate them 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	The bone marrow was not thourouly rinsed	The bone marrow should be throughly flushed out as much as possible until the bone cavity appears white to the naked eye
	Shortage of tissue sampling amount	If a significant amount of tissue is discarded due to bone cutting or breaking bones, the number of rats can be increased appropriately
	Cell differential adhesion time is more than 18 hours	Perform differential adhesion strictly according to the differential time in the manual
Slow cell adhesion	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 preparing it for more than three months
	Over-aged rat	Use rats aged 20-30 days postnatally. Cells isolated from rats of varying ages may exhibit differences in their adhesion capabilities. You could attempt to conduct centrifugation and replace the medium, then continue culturing for additional days.
Low cell purity	Muscle tissue was not completely removed and fell into the bone marrow irrigation fluid	Ensure the muscle tissue is completely removed
	The bone was broken, exposing bone marrow fluid within the muscle tissue, but it was still used	In this case, the bone tissue must be discarded and the quantity of cells can be enhanced by using more rats
	The differential adhesion time is less than 18 hours	Perform differential adhesion strictly according to the differential time in the manual

Anatomy Images for Reference

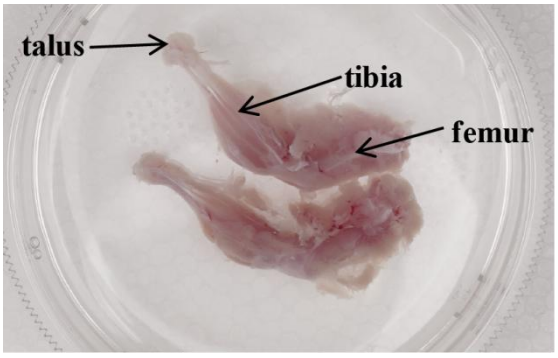


Figure 1. Get the intact femur and tibia.

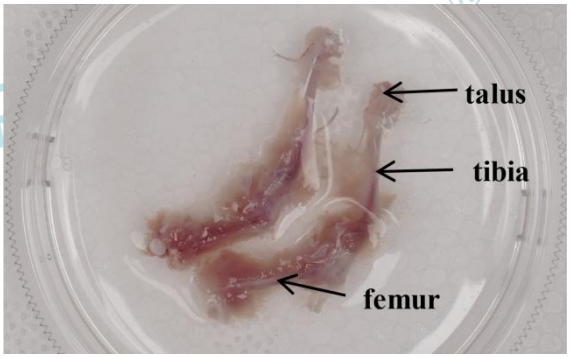


Figure 2. Remove large pieces of muscle tissue and don't need to clean them up very well .

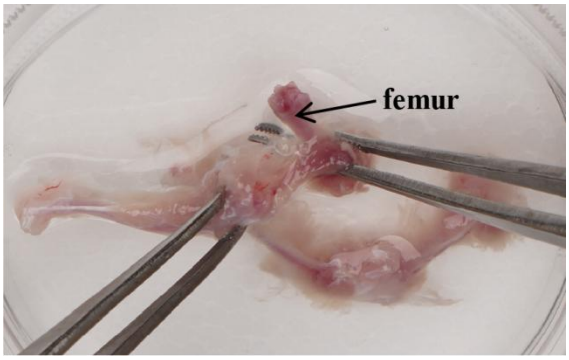


Figure 3a. Break apart forcefully aganist the direction of joint movement .



Figure 3b. Break apart forcefully aganist the direction of joint movement .

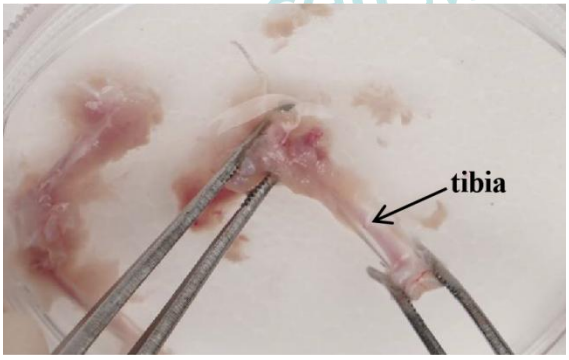


Figure 3c. Break apart forcefully aganist the direction of joint movement .



Figure 4. Separate the intact femur and tibia.

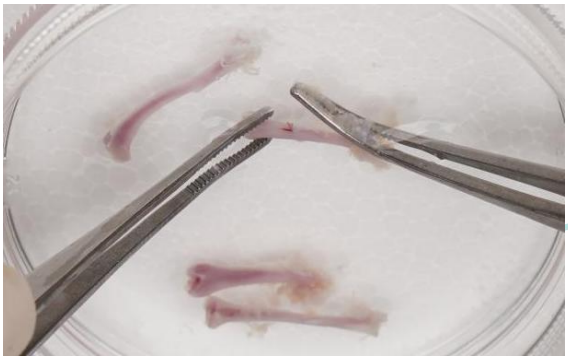


Figure 5. Remove the residual muscle tissue on the surface of femur and tibia.



Figure 6. Pure femur and tibia.



Figure 7a. Cut off both ends of the bone.

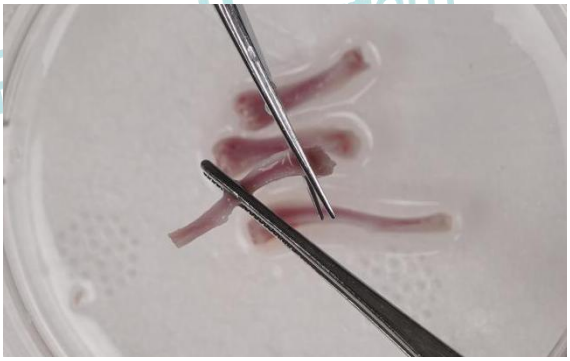


Figure 7b. Cut off both ends of the bone.



Figure 8. Aspirate the Specialized Washing Solution.



Figure 9. Stir the needle and rinse the bone marrow.



Figure 10. The bone turns white and translucent.

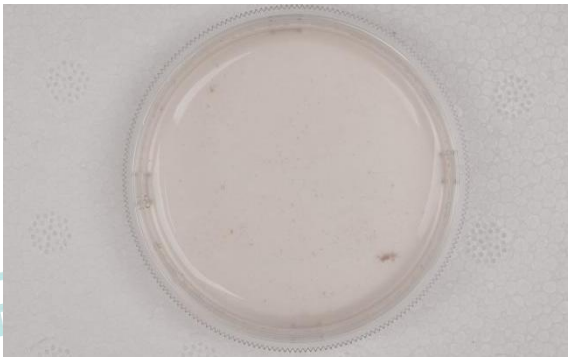


Figure 11. Collect the bone marrow fluid in the culture dish.

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