Rat Bone Marrow-Derived Macrophage Isolation and Culture Kit

Cat. No.: P-CA-617

Size: 3Tests / 10Tests

Background

The Rat Bone Marrow-Derived Macrophage Isolation and Culture Kit is specifically developed for the extraction of primary rat bone marrow-derived Macrophages. 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. The cell belongs to the group of terminally differentiated cells, which is not a proliferating cell group. It is recommended to inoculate directly into the corresponding culture vessel according to the experimental requirements after separation. Through immunofluorescence analysis, the cell purity (CD68 positive rate) has been confirmed to exceed 90%.

Scope of Application

This product is suitable for extracting Rat Bone Marrow-Derived Macrophages from 20-30-day-old rats of various strains, such as Wistar and SD. After the processes of tissue isolation and plating for 3-4 days, a yield of $>1\times10^6$ cells can be obtained.

Note: The intact tibia and femur tissue extracted from 1 rat, typically yields 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 Macrophages	3Tests (250 mL) 10Tests (500 mL×2)	Pale Yellow Clear Liquid	2-8°C, 1 year
Specialized Lysis Solution For Rat Bone Marrow-Derived Macrophages	3Tests (15 mL) 10Tests (50 mL)	Colourless Clear Liquid	2-8°C, 1 year
Basic Culture Medium For Rat Bone Marrow-Derived Macrophages	3Tests (100 mL) 10Tests (300 mL)	Red Clear Liquid	2-8°C, 1 year
Supplement For Rat Bone Marrow-Derived Macrophages	3Tests (10 mL) 10Tests (30 mL)	Yellow Clear Liquid	-5~-20°C, 1 year
70 μm Cell Filter	3 Tests (3 pcs) 10 Tests (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 Macrophages) 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 Macrophages: Thaw at 4°C and equilibrate to room temperature.
   ② Specialized Washing Solution For Rat Bone Marrow-Derived Macrophages & Basic Culture Medium For Rat Bone Marrow-Derived Macrophages & Specialized Lysis Solution For Rat Bone Marrow-Derived Macrophages: Equilibrate to room temperature.
3) Preparation of Complete Culture Medium: Add 10 mL of Supplement For Rat Bone Marrow-Derived Macrophages into 100 mL of Basic Culture Medium For Rat Bone Marrow-Derived Macrophages, 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 bones. Place them into the petri dish and add 10 mL of Specialized Washing Solution For Rat Bone Marrow-Derived Macrophages (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 petri dish containing 10 mL of Specialized Washing Solution For Rat Bone Marrow-Derived Macrophages.

③ Bone Tissue Dissection:

a. Use Straight Forceps 3 to fix the tissue, and 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 Macrophages 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 the 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, add 10 mL of Specialized Washing Solution For Rat Bone Marrow-Derived Macrophages.

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 Macrophages. 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 petri dish and add 10 mL of the Specialized Washing Solution For Rat Bone Marrow-Derived Macrophages. 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 Macrophages from the petri dish (Figure 8). Insert the needle into the thicker end of the bone and perform above the petri 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 petri 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 Macrophages into the filter to collect the bone marrow cells on the filter. Then transfer the filtrate into 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 1500 rpm for 5 mins. Discard the supernatant and retain the cell pellet. Add 5 mL of Specialized Lysis Solution for Mouse Bone Marrow-Derived Macrophages 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 Macrophages. 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: Take out the culture vessel or T25 cell culture flask for the next experiment, and resuspend the cell pellet in the centrifuge tube with 10 mL of Complete Culture Medium For Rat Bone Marrow-Derived Macrophages. Then inoculate cell suspention into the culture vessel. Incubate at 37°C, 5% CO_2.

2) Medium Renewal: Perform the first medium change at Day 3. Observe under the microscope, if the confluence of adherent cells is around 80%, discard the supernatant directly and add fresh compelet culture medium to the vessel. If most of the cells are not attached, collect the supernatant and centrifuge, resuspend cell pellets with fresh complete culture medium then add them back to the culture vessel. Followed by subsequent replacements every 2-3 days. Cells typically reach 80-90% confluency within 3-4 days post-seeding.

3) Cell Subculture: It belongs to the group of terminally differentiated cells and does not proliferate. Therefore, it is recommended to inoculate the cells into the plate according to the experiment requirements after isolation. Minimize digestion unless absolutely necessary.

4) Digestion Method:

① Aspirate the culture medium from the T25 cell culture flask and wash the cells once with PBS. Add 1 mL of lidocaine (12 mM) digestive solution to the culture flask, gently rotate the flask to ensure the digestive solution covers the entire bottom of the flask, and incubate at 37°C for 3 mins (preferably not exceeding 5 mins). Observe under an inverted microscope until the cells have retracted and become round. Then, add 5 mL of complete medium to dilute the digestive solution. Gently mix and disperse the cells using a pipette, aspirate the cell suspension, and centrifuge at 1200 rpm for 5 minutes. Then, discard the supernatant, resuspend the cells in complete medium and perform cell counting. Inoculate cell suspention into the appropriate experimental vessels. Once the cells are fully adhered to the wall, culture and observe them for experimental use. Afterward, replace the culture medium with fresh complete culture medium

according to the specified frequency.

② If the cells cannot be digested by lidocaine, replace the digestive solution with 0.25% trypsin and operate according to the above digestion method. If the cells still cannot be digested, add 3 mL complete culture medium to terminate the digestion and use sterile cell scraping to directly scrape the cells (this method is not recommended unless cells cannot be digested and collected, as cell viability can decrease due to mechanical damage).

Troubleshooting

Problem	Possible Cause	Solution
Low yield/low viability	The bone marrow was not thourouly rinsed	The bone marrow should be throughly flushed out 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 cutting or breaking bones, the number of rats can be increased appropriately
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.

Anatomy Images for Reference

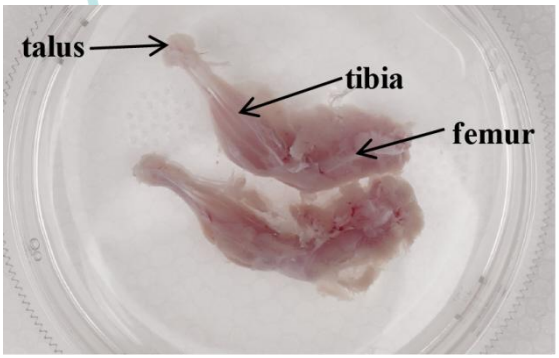

Figure 1. Get the intact femur and tibia.

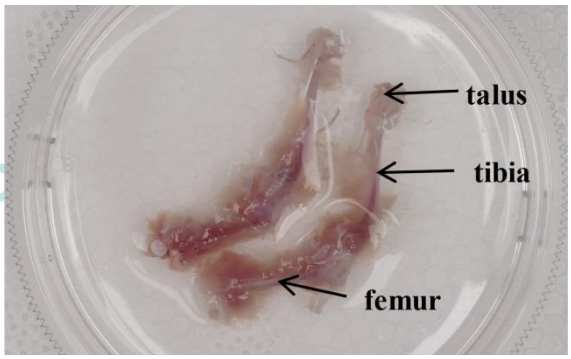

Figure 2. Remove large pieces of muscle tissue and don't need to clean them up very well.

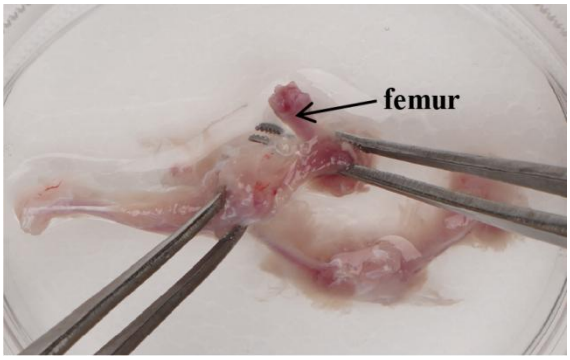

Figure 3a. Break apart forcefully aganist the direction of joint movement.

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

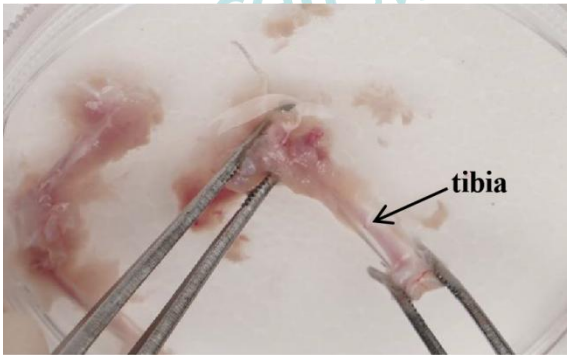

Figure 3c. Break apart forcefully aganist the direction of joint movement.

Figure 4. Separate the intact femur and tibia.

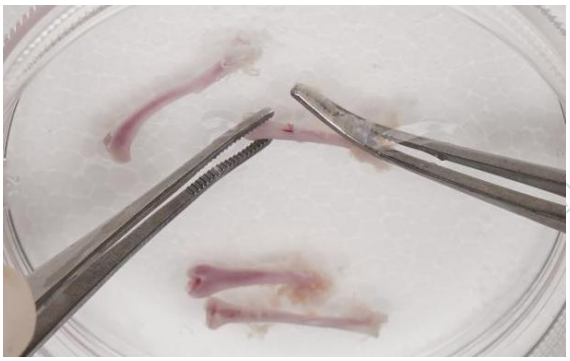

Figure 5. Remove the residual muscle tissue on the surface of femur and tibia.

Figure 6. Pure femur and tibia.

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

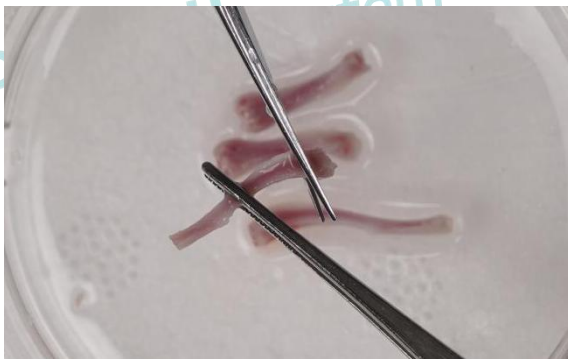

Figure 7b. Cut off both ends of the bone.

Figure 8. Aspirate the Specialized Washing Solution.

Figure 9. Stir the bone marrow with the needle and flush it.

Figure 10. The bone turns white and translucent.

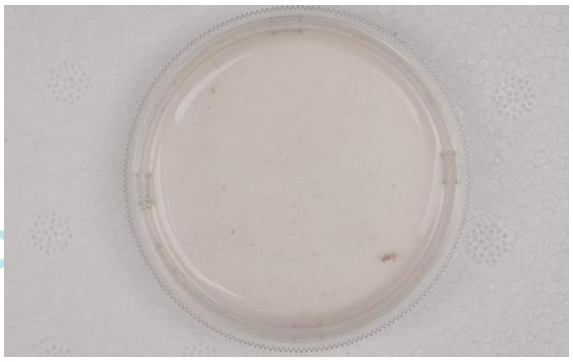

Figure 11. Collect the bone marrow fluid in the culture dish.

Procell system®
Procell system®
Procell system®