Rat Bone Marrow Mononuclear Cells Isolation and Culture Kit

Cat. No.: P-CA-619

Size: 3Tests/10Tests

Background

The Rat Bone Marrow Mononuclear Cells Isolation and Culture Kit is specifically developed for the extraction of primary Rat bone marrow mononuclear cells. Verified through standardized procedures, each Test of this kit supports the acquisition of one flask of cells (T-25 culture flask), with a cell count exceeding 1×10^7 cells.

Scope of Application

This product is suitable for extracting Rat Bone Marrow Mononuclear Cells from 20-30-day-old rats of various strains, such as Wistar and SD. Following standard tissue isolation and plating procedures, it can yield $>1\times10^7$ cells..

Note: The intact tibia and femur tissue extracted from 3 rats,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 Mononuclear Cells	3Tests (250 mL) 10Test (500 mL×2)	Pale Yellow Transparent Liquid	2-8℃, 1 year
Specialized Isolation Solution for Rat Bone Marrow Mononuclear Cells	3Tests (15 mL) 10Tests (50 mL)	Yellow Transparent Liquid	2-8℃, 1 year
Basic Culture Medium for Rat Bone Marrow Mononuclear Cells	3Tests (100 mL) 10Tests (300 mL)	Red Transparent Liquid	2-8℃, 1 year
Supplement for Rat Bone Marrow Mononuclear Cells	3Tests (10 mL) 10Tests (30 mL)	Yellow Transparent Liquid	-5~-20℃, 1 year
70 μm Cell Filter	3Tests (3 pcs) 10Tests (10 pcs)	Orange	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 to -20℃ (such as Supplement for Rat Bone Marrow Mononuclear 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℃ before use to avoid repeated freeze-thaw cycles.

Precautions

1. 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.
2. 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 (At least 3 pairs of ophthalmic scissors, 3 pair 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 Mononuclear Cells: Thaw at 4℃ and equilibrate to room temperature.

② Specialized Washing Solution forRat Bone Marrow Mononuclear Cells & Basic Culture Medium for Rat Bone Marrow Mononuclear Cells&Specialized Isolation Solution for Rat Bone Marrow Mononuclear Cells: Equilibrate to room temperature.

3) Preparation of Complete Culture Medium: Add 10 mL of Supplement for Rat Bone Marrow Mononuclear Cells to 100 mL of Basic Culture Medium for Rat Bone Marrow Mononuclear Cells, then 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 Euthanasia and Disinfection Protocol: 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 disinfection, 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 on 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: The entire leg was exposed, while the caput femoris and the calcaneus (ankle bone at the heel) remained uncovered by fur.

b. Using Curved Forceps 2 with the left hand to hold the ankle bone, cut the ankle bone and metatarsal bone with Ophthalmic Scissors 2, lift the ankle bone, use Ophthalmic Scissors 2 to cut the muscle and skin behind the leg to the joint at the root of the thigh, and cut the joint to get the complete femur and tibia.Transfer the specimen to a culture dish and add 10 mL of Specialized Washing Solution for Rat Bone Marrow Mononuclear 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 cut off the tibia and femur. If cut off, bone marrow is exposed and bone marrow cavity contacts with muscle. The tissue should be discarded to prevent tissue 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 Perform tissue dissection using this set of new Ophthalmic Scissors and Curved Forceps. Rinse the tissue once and transfer it into a new culture dish containing 10 mL of Specialized Washing Solution for Rat Bone Marrow Mononuclear Cells.

③ Bone Tissue Dissection:

a. Use straight forceps 3 to fix the tissue, and the muscle tissue was loosened by bending forceps 3. Pull off large pieces of muscle tissue and avoid thorough cleaning (Figure 2). The bone was placed into a new culture dish, and 10 mL of Specialized Washing Solution for Rat Bone Marrow Mononuclear Cells was added to wash it back and forth.

b. Use straight forceps 3 and curved forceps 3 to grasp the lateral bone of the knee joint , and forcefully pry it apart in the direction opposite to joint movement (Figure 3). Be careful not to break the bone, and separate the complete 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 Mononuclear Cells. (Figure 4).

c. Use straight forceps 3 and curved forceps 3 to remove the residual muscle tissue on the surface of femur and tibia (Figure 5), and keeping the bone intact, and leaving the pure femur and tibia (Figure 6). Transfer the tissue to a new culture dish, add 10 mL of Specialized Washing Solution for Rat Bone Marrow Mononuclear Cells, and store for later use.

d. Bones were picked up one by one using curved forceps 3, and the two ends of the bone were cut off with ophthalmic scissors 3 to expose the bone marrow (Figure 7), which was placed in a dry sterile culture dish.

e. Prepare a new culture dish and add 10 mL of the Specialized Washing Solution for Rat Bone Marrow Mononuclear Cells. Use the left hand to pick up a bone with a curved forceps, and take a 2 mL syringe with your right hand to draw the Specialized Washing Solution for Rat Bone Marrow Mononuclear Cells from the culture dish (Figure 8). Insert a needle into the thick end of the bone on the culture dish, being careful not to let the bone fall into the dish. Stir the bone marrow with the needle and flush it (Figure 9) until the bone turns white and translucent (Figure 10). Collect the bone marrow fluid in the culture dish (Figure 11) and gently aspirate about 15 times with a 5 mL pipette or a Bacto 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 washing solution.

② 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, use a clean pipette tip to slowly add 2 mL of Specialized Washing Solution for Rat Bone Marrow Mononuclear Cells over the filter to collect the bone marrow suspension on the filter. Collect the filtrate in a 15 mL centrifuge tube.

Note: If filtration is impeded, slightly tilt the filter to reduce vacuum seal between the filter and the tube rim.

③ Take a 15 mL centrifuge tube and centrifuge at 1500 rpm for 5minutes; Discard the supernatant and retain the cell pellet.Take a new 15 mL centrifuge tube and add 4 mL of Specialized Isolation Solution for Rat Bone Marrow Mononuclear Cells. Using a 200 μL pipette gun, slowly add 3 mL of PBS-resuspended cell suspension above the level of 4 mL of Specialized Isolation Solution for Rat Bone Marrow Mononuclear Cells to form a density gradient isolate. The centrifuge tubes are then subjected to a 25-minutes centrifugation at 1500 g, with the acceleration and deceleration set to 1 gear. The cells at the interface between the PBS and the separation liquid are aspirated and collected in a 15 mL centrifuge tube. PBS is added to adjust the total volume to 13 mL, and the mixture is centrifuged at 1800 rpm for 5 minutes. The supernatant is discarded, and the pellet is retained.

4. Cell Culture and Subculture

1) Cell Seeding: Take out the culture dish or T25 culture flask for the next experiment, and resuspend the cell pellet in the centrifuge tube with 10 mL of Complete Culture Medium of Rat Bone Marrow Mononuclear Cells, then inoculat into the culture dish. The cells were cultured in a incubator at 37℃, 5% CO_2.

2) Medium Renewal: Perform the first medium change at 48 hours.and Subsequent medium replacements are performed on the third day, the fifth day, and then every 2-3 days. After inoculation for about 3-4 days, cell confluence will reach 80-90%.

3) Cell Subculture: No proliferation; no passage.

Troubleshooting

Problem	Possible Cause	Solution
Low yield/low viability	The bone marrow was not washed clean	The bone marrow should be washed out as much as possible until the bone cavity is white to the naked eye
	hortage of tissue sampling amount	If more tissue is discarded due to bone cutting or breaking, the number of rats can be increased appropriately
The cell adheres slowly	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
	The age of the Rat is inappropriate	Use Rat aged 20-30 days postnatally. If cells do not adhere to the wall due to different days of age, centrifuge and change the culture for several days
Low cell purity	The muscle tissue was not cleaned up and fell into the bone marrow lavage fluid	Ensure the muscle tissue are cleaned out
	The bone is broken and the bone marrow fluid is exposed in the muscle tissue, and it's still used	In this case, the bone tissue must be discarded, and the number of cells can be increased by using more rats

Anatomy Images for Reference

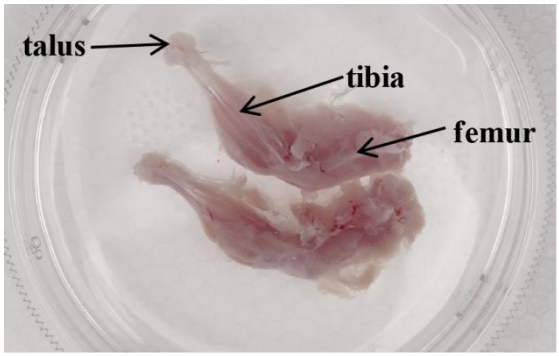

Figure 1.Get the complete femur and tibia.

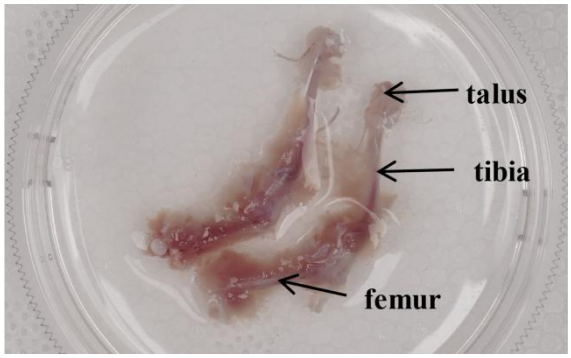

Figure 2. Pull off large pieces of muscle tissue and avoid thorough cleaning .

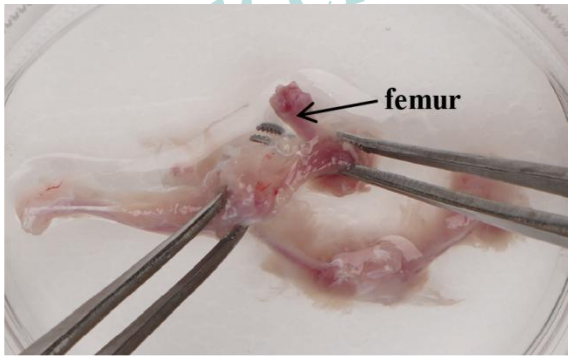

Figure 3a. Pry it apart in the direction opposite to joint movement

Figure 3b. Pry it apart in the direction opposite to joint movement .

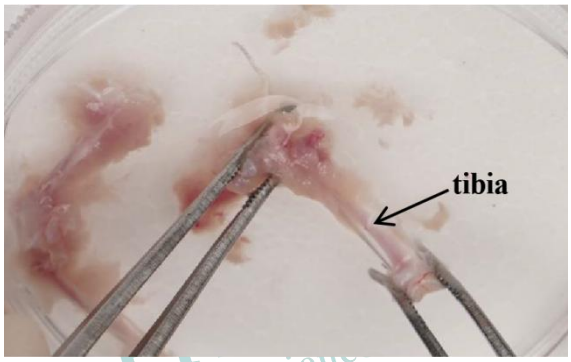

Figure 3c. Pry it apart in the direction opposite to joint movement **.**

Figure 4. Separate the complete femur and tibia.

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

Figure 6. Pure femur and tibia.

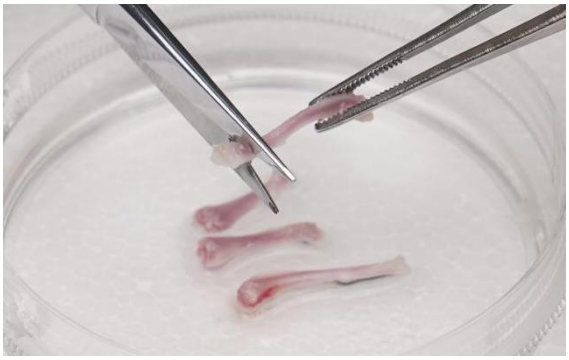

Figure 7a.Cut off both ends of the bone.

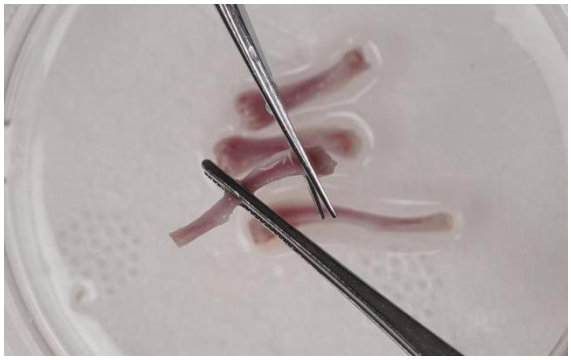

Figure 7b. Cut off both ends of the bone.

Figure 8.Draw the Specialized Washing Solution.

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

Figure 10.The bone turns white and translucent.

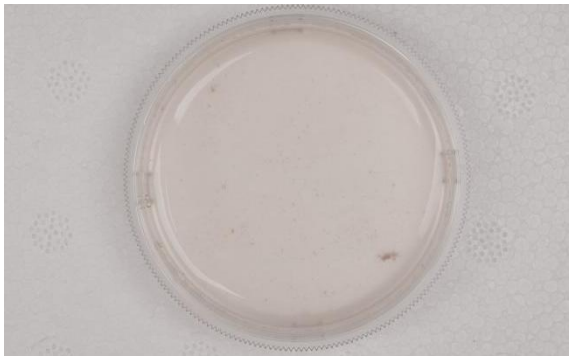

Figure 11.Collect the bone marrow fluid in the culture dish.