Mouse Articular Chondrocyte Isolation and Culture Kit

Cat. No.: P-CA-717

Size: 3Tests / 10Tests

Background

The Mouse Articular Chondrocyte Isolation and Culture Kit is specifically developed for the extraction of primary Mouse Articular Chondrocytes. As validated, standard operation using this kit enables the acquisition of one flask of cells (T-25 culture flask) per 1 Test, with a cell count exceeding 1×10^6 cells. When subcultured at a 1:2 ratio, the cells can undergo 3-5 passages, with the best cell state within the first 3 passages. Through immunofluorescence analysis, the cell purity (Collagen II positive rate) has been confirmed to exceed 90%.

Scope of Application

This product is suitable for extracting Mouse Articular Chondrocytes from 14-day-old mice of various strains (e.g., KM, C57, Balb/C). After processes of tissue isolation, enzymatic digestion, and 48-hour plating, a yield of $>1\times10^6$ cells can be obtained.

Note: 6 articular cartilage tissue extracted from 3 mice typically yield enough cells for one T-25 flask. The exact number of mice required may vary depending on the size and quantity of saphenous vein tissue harvested during this procedure. If the amount of tissue obtained is insufficient, additional experimental mice may be needed to prevent cell quantity deficiency.

Kit Components

Name	Size	Appearance	Storage and Expiration Date
Specialized Washing Solution for Mouse Articular Chondrocytes	3Tests (250 mL) 10Tests (500 mL×2)	Pale Yellow Clear Liquid	2-8°C, 1 year
Specialized Digestive Solution for Mouse Articular Chondrocytes	3Tests (15 mL) 10Tests (50 mL)	Yellow Clear Liquid	-5~-20°C, 1 year
Basic Culture Medium for Mouse Articular Chondrocytes	3Tests (50 mL) 10Tests (100 mL)	Red Clear Liquid	2-8°C, 1 year
Supplement for Mouse Articular Chondrocytes	3Tests (10 mL) 10Tests (20 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
100 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 Specialized Digestive Solution for Mouse Articular Chondrocytes) 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 mice to familiarize yourself with operational procedures and improve tissue isolation

efficiency.

3. Reagent preparation or aliquoting must strictly adhere to aseptic techniques protocols. 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, 1 pair of straight forceps, 2 pairs of curved forceps, 1 pair of micro straight forceps, 1 pair of micro curved forceps,1 pair of scalpel and blade), 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 Specialized Digestive Solution for Mouse Articular Chondrocytes & Supplement for Mouse Articular Chondrocytes: Thaw at 4°C and equilibrate to room temperature.
   ② Specialized Washing Solution for Mouse Articular Chondrocytes & Basic Culture Medium for Mouse Articular Chondrocytes: Equilibrate to room temperature.
3) Preparation of Complete Culture Medium: Add 10 mL of Supplement for Mouse Articular Chondrocytes into 50 mL of Basic Culture Medium for Mouse Articular Chondrocytes, 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 -5~-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 animal 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, Ophthalmic Scissors 3 and Curved Forceps 3).

   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 Mouse Fixation: Secure the mouse in a supine position within the clean bench using needles, preparing for tissue harvesting.
   ③ Tissue Harvesting Procedure:
      a. Use the straight forceps 1 to lift the mouse's leg skin, then make an incision along the lifted area with ophthalmic scissors (Fig.1).

      Note: Ensure the skin is fully exposed from the abdomen to 2 cm below the knee joint, avoid hair contamination by tearing the fur away from the dissection area.

b. Identify the white patella at the knee joint.Use the straight forceps 1 in the left hand to assist in fixing the mouse's leg. Use the scalpel in the right hand to circumferentially incise around the patella.Grasp the patellar ligament near the tibia with curved forceps 2, then tear the connective tissue toward the femur to expose the joint cavity (Fig.2). Clear residual connective tissue within the cavity.

c. Use the straight forceps 1 in the left hand to assist in fixing the rat's leg. With a scalpel in the right hand, excise cartilage along the curvature between the femur and knee joint, following the interface of white cartilaginous tissue and underlying bone (Fig.3).Transfer the cartilage to a culture dish using straight forceps 2 (Fig.4), then add 10 mL of Mouse Articular Chondrocyte-Specific Washing Solution.

Note: Cartilage appears whitish translucent and elastic. If hard structures (bone) are encountered, withdraw the blade and re-cut along the cartilage curvature.

3. Tissue Processing and Digestion

1) Tissue Processing

① Put Micro Straight Forceps on the EP tube rack within the clean bench, ensuring the distal third of each tool suspended.

② Holding micro straight forceps in the left hand and a scalpel in the right hand to manipulate the cartilage tissue: Rinse the harvested cartilage tissue once to remove excess blood stains and connective tissue, then transfer the tissue to a new culture dish containing 10 mL of Specialized Washing Solution for Mouse Articular Chondrocytes.

③ Hold the tissue with micro straight forceps in the left hand, and excise the cartilage margins along with adherent adipose tissue, synovial membrane, fascia, and any inadvertently removed bone tissue using a scalpel in the right hand, retaining only the translucent white pure cartilage tissue.

Note: Cartilage tissue exhibits inherent elasticity; pure cartilage can be distinguished by scraping with forceps or a scalpel.

④ Aspirate and discard the Washing Solution for Mouse Articular Chondrocytes from the culture dish, hold the tissue steady with curved forceps 3 in the left hand, and mince each piece of articular cartilage tissue into 4-6 uniform-sized fragments using a surgical scalpel in the right hand.

2) Tissue Digestion

① Add 5 mL of Specialized Digestive Solution for Mouse Articular Chondrocytes into a new culture dish. With the micro curved forceps in the right hand, transfer the fragmented articular cartilage tissue pieces into the dish containing the Specialized Digestive Solution for Mouse Articular Chondrocytes. Gently pipette the tissue fragments up and down to disperse them, then place the dish in a 37°C incubator for a 48-hour.

② After digestion, take out the dish from the incubator and use a 5 mL pipette or a Pasteur pipette to Pipette the suspension approximately 30 times.

③ Place a 100 μm cell strainer and a 70 μm cell strainer onto the mouth of 2 new 50 mL centrifuge tube. Rinse both strainers separately using 3-5 mL of Specialized Washing Solution for Mouse Articular Chondrocytes. Then, carefully aspirate the tissue digestion solution from Step 2 using a pipette, and filter it sequentially through the 100 μm and 70 μm cell strainers. After filtration, slowly add 3-5 mL of the Specialized Washing Solution for Mouse Articular

Chondrocytes to the upper surface of the strainers using a clean pipette tip. Collect the filtrate in the 50 mL centrifuge tube.

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

④ The collected filtrate was transferred to a 15 mL centrifuge tube and centrifuged at 1200 rpm for 5 minutes; the supernatant was discarded while retaining the pellet.

⑤ Subsequently, 5 mL of Specialized Washing Solution for Mouse Articular Chondrocytes was added to the tube to resuspend the pellet. The resulting cell suspension was then transferred to a new 15 mL centrifuge tube and centrifuged again at 1200 rpm for 5 minutes

4. Cell Culture and Subculture

1) Cell Seeding: Take out the T25 cell culture flask, and resupend the cell pellet in 5 mL of Complete Culture Medium of Mouse Articular Chondrocytes and transfer the suspension into the T25 cell culture flask. The cells were cultured in a incubator at 37°C, 5% CO_2. After 3 to 5 days of culture, cell confluence reaches approximately 80%.

2) Cell Subculture: When the cell confluence reaches 80-90%, it is ready for passaging. First, aspirate and discard the old medium from the T25 cell culture flask and wash the cells once with 2-3 mL of PBS. Then, add 1mL of 0.25% trypsin digestive solution to the T25 flask, gently rotate the flask until the digestive solution covers the entire bottom, then aspirate and disgard the excess trypsin solution, incubate at 37°C for 1-3 min. Next, observe under an inverted microscope until the cells retract and become rounded, then add 5mL of Complete Culture Medium for Mouse Articular Chondrocytes to terminate the digestion. Resuspend and disperse the cells by gently pipetting with a 5 mL pipette or Pasteur pipette. Inoculate the cells into new culture vessels according to the desired 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	Insufficient dissociation	Check the storage conditions of the digestive solution to ensure it has not been stored at 4°C for more than 30 days
		Ensure the tissue quantity matches the kit requirements
		Ensure that the tissue is gently and adequately pipetted up and down.
	Over-digestion	Avoid fragmenting the tissue blocks excessively.
Slow cell growth	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
	Improper subculturing ratio	When passaging at 1:2 ratio, calculate based on the vessel surface area to maintain proper cell seeding density
	Over-passaged	Limit cell passage to 3-5 times to prevent a slowdown in proliferation.
Low cell purity	The outer membrane layer of the tissue was not completely removed	Ensure that the final digested tissue is pure white cartilage tissue. If unsure, scissors or tweezers can be used for compression. Cartilage tissue has a certain elasticity and toughness, and it will not be particularly hard when pressed, but there will be some resistance. Does not contain pink, red bones or white connective tissue, muscles, etc

Continued

Improper age of Mice	Mice are too old or too young	Using excessively young mice may cause difficulties in tissue harvesting, resulting in insufficient tissue quantity and low cell yield . Conversely, using older mice may lead to endochondral ossification , where cartilage matures into bone, thereby reducing available tissue and making cell isolation unfeasible . Based on laboratory experience, 14-day-old mice are recommended as the optimal model for experimentation. Cells can be reliably obtained from 2- to 4-week-old mice,and older mice should be avoided due to diminished experimental viability.
Cells appear morphologically round during initial adherence	Normal phenomenon	If the cells appear rounded after 2-3 days of adherence, they should be passaged normally at a 1:2 ratio. After passaging, the cell morphology will return to normal.

Anatomy Images for Reference

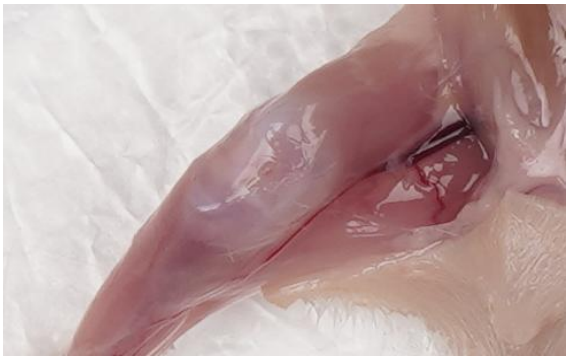


Figure 1. Peel the skin of legs and abdomen

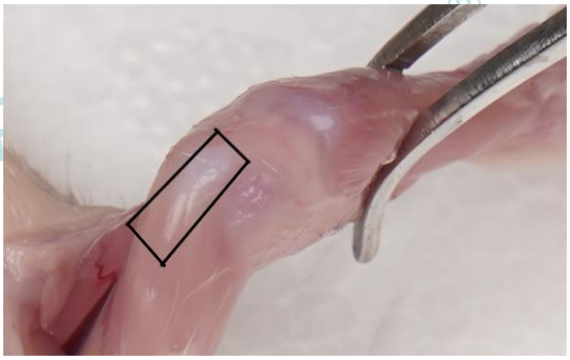


Figure 2a. Locate the patella and cut around the patella to expose the articular cavity

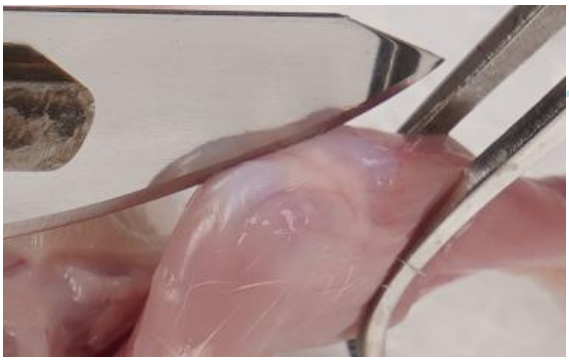


Figure 2b. Locate the patella and cut around the patella to expose the articular cavity



Figure 2c. Locate the patella and cut around the patella to expose the articular cavity

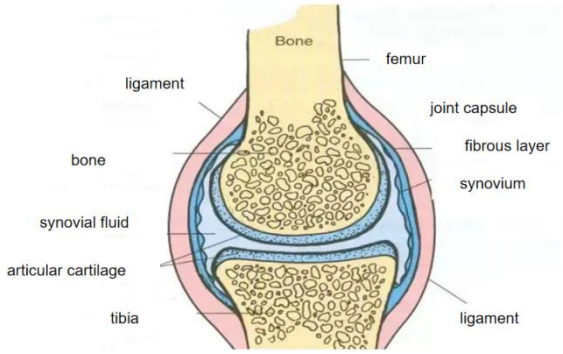


Figure 3. Schematic diagram of articular cartilage tissue



Figure 4a. Using scalpel to excise the articular cartilage tissue.

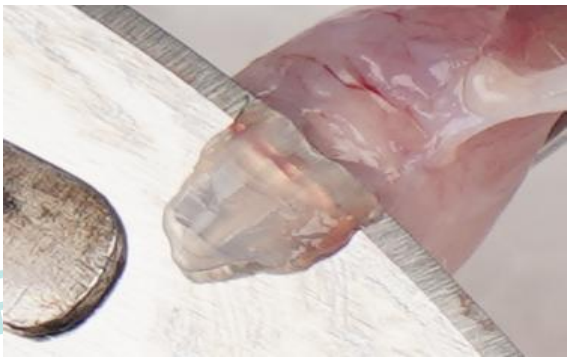


Figure 4b. Using scalpel to excise the articular cartilage tissue

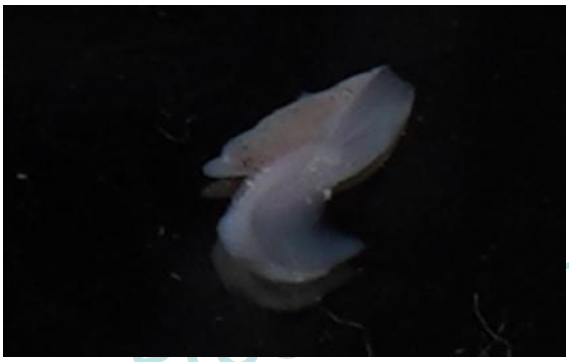


Figure 5. Excised articular cartilage tissue

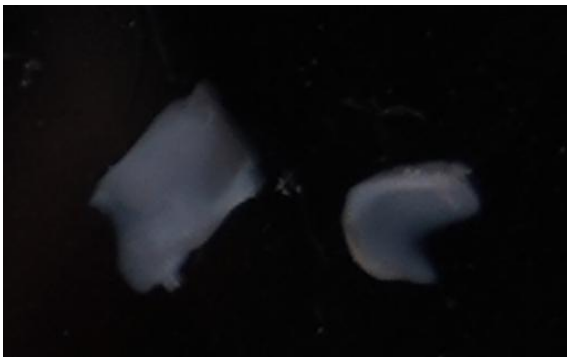


Figure 6. Cleaned .articular cartilage tissue