Rat Acetabular Chondrocyte Isolation and Culture Kit

Cat. No. : P-CA-624

Size : 3Tests / 10Tests

Background

The Rat Acetabular Chondrocyte Isolation and Culture Kit is specifically developed for the extraction of primary Rat Acetabular 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 Rat Acetabular Chondrocytes from 14-day-old rats of various strains (e.g., Wistar, SD). After processes of tissue isolation, enzymatic digestion, and 48-hour plating, a yield of > 1×10^6 cells can be obtained.

Note: 6 articular cartilage tissues 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 saphenous vein 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 Acetabular Chondrocytes	3Tests (250 mL) 10Test (500 mL×2)	Pale Yellow Clear Liquid	2-8°C, 1 year
Specialized Digestive Solution for Rat Acetabular Chondrocytes	3Tests (15 mL) 10Tests (50 mL)	Yellow Clear Liquid	-5~-20°C, 1 year
Basic Culture Medium for Rat Acetabular Chondrocytes	3Tests (50 mL) 10Tests (100 mL)	Red Clear Liquid	2-8°C, 1 year
Supplement for Rat Acetabular 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)	Orange	Room temperature, 3 years
100 μm Cell Filter	3Tests (3 pcs) 10Tests (10 pcs)	Green	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°C (such as Specialized Digestive Solution for Rat Acetabular Chondrocytes) can be preserved at 4°C for 30 days after thawing. For long-term storage, aliquot them into single-use portions and store at -20°C. Thaw again at 4°C 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 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 scissors), 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 Rat Acetabular Chondrocytes & Supplement for Rat Acetabular Chondrocytes: Thaw at 4°C and equilibrate to room temperature.
   ② Specialized Washing Solution for Rat Acetabular Chondrocytes & Basic Culture Medium for Rat Acetabular Chondrocytes: Equilibrate to room temperature.
3) Preparation of Complete Culture Medium: Add 10 mL of Supplement for Rat Acetabular Chondrocytes into 50 mL of Basic Culture Medium for Rat Acetabular Chondrocytes, mix thoroughly.

   Note: Storage conditions for complete culture medium: 2-8°C, 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°C 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, 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 Rat Fixation: Secure the rat in a supine position within the clean bench using needles, preparing for tissue harvesting.
   ③ Tissue Harvesting Procedure:
      a. Use straight forceps 1 to lift the skin of rat's leg and abdomen, then cut along the grasped portion of the leg and abdominal skin until reaching the thoracic region make an incision along the lifted area with ophthalmic scissors.

         Note: Ensure complete exposure of the skin from abdomen to 2 cm below the knee joint. Tear the fur away from the dissection area to prevent contamination.

      b. Anatomical Position of Acetabulum: The acetabulum is situated centrally on the lateral surface of the hip bone, presenting as a hemispherical socket. It is formed by the fusion of three bones: the ilium, ischium, and pubis. Its opening faces anteroinferiorly and laterally, with the articular surface covered by approximately 2 mm-thick hyaline cartilage. Key landmark: The circumferential labrum at the acetabular rim deepens the socket, enhancing femoral head coverage. This structure serves as a crucial landmark during cartilage dissection (see Figure 1).
      c. Locate the rat knee joint. Hold the thigh muscle tissue with Curved Forceps 2 in the left hand, and use Ophthalmic Scissors 2 in the right hand to incise along the thigh muscle to the groin. Trim excess muscle tissue to expose the hip joint region.
      d. Option 1: Hold the rat femur with Curved Forceps 2 in the left hand. Use Ophthalmic Scissors 2 in the right hand to sever the connections between the hip joint and the superior/lateral ilium. Clamp and extract the acetabular cartilage with Curved Forceps 3, then place it in a culture dish containing 10 mL of Specialized Washing Solution for Rat

Acetabular Chondrocytes.

Option 2: Dislocate the hip joint using Curved Forceps 2 and Ophthalmic Scissors 2 while preserving the intact femoral head cartilage cap. The acetabular fossa (where the femoral head connects to the body) is then excised with Ophthalmic Scissors 2. Clamp and extract the acetabular cartilage with Ophthalmic Scissors 3, placing it in a Petri dish with 10 mL of Specialized Washing Solution for Rat Acetabular Chondrocytes.

Note: The hip joint is the connection point between the trunk and the thigh. If beginners are unable to identify the location of the hip joint, they can use curved forceps to grasp the rat's femur. By moving the femur, the connection point between the femur and the trunk can be observed, which is the hip joint.

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 clear excess blood clots and connective tissue. Transfer the tissue to a new Petri dish containing 10 mL of specialized cleaning solution for rat acetabular chondrocytes.

③ Fix the hip joint with micro straight tissue forceps in the left hand, and use ophthalmic scissors 3 in the right hand to excise non-cartilaginous structures (e.g., other bones and connective tissue) surrounding the white acetabular cartilage. Retain the pure white acetabular cartilage tissue, and transfer it to a new culture dish containing 10 mL of Specialized Washing Solution for Rat Acetabular Chondrocytes.

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

④ Aspirate the Specialized Washing Solution for Rat Acetabular 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 Rat Acetabular Chondrocytes into a new culture dish. Using curved micro forceps held in the right hand, transfer the fragmented articular cartilage tissue pieces into the dish containing the Specialized Digestive Solution for Rat Acetabular Chondrocytes.Gently pipette the mixture to suspend the tissue fragments, 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 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 Rat Acetabular 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 Rat Acetabular 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 Rat Acetabular 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 resuspend the cell pellet in the centrifuge

tube with 5 mL of Complete Culture Medium of Rat Acetabular Chondrocytes, then inoculat 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 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 Rat Acetabular 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 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	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 organization 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
Improper age of Rats	Rats are too old or too young	Using excessively young rats may cause difficulties in tissue harvesting, resulting in insufficient tissue quantity and low cell yield. Conversely, using older rats 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 rats are recommended as the optimal model for experimentation. Cells can be reliably obtained within 2-4 weeks, and older rats 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

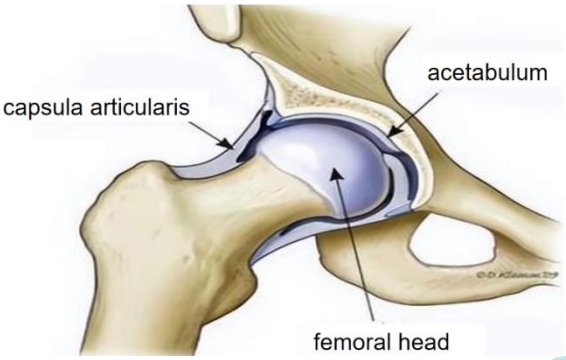

Figure 1a. Structural picture of human acetabular region

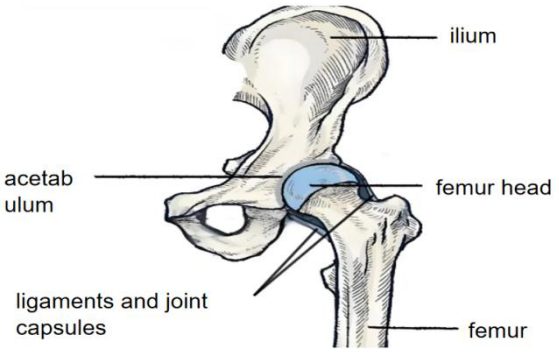

Figure 1b. Structural picture of human acetabular region

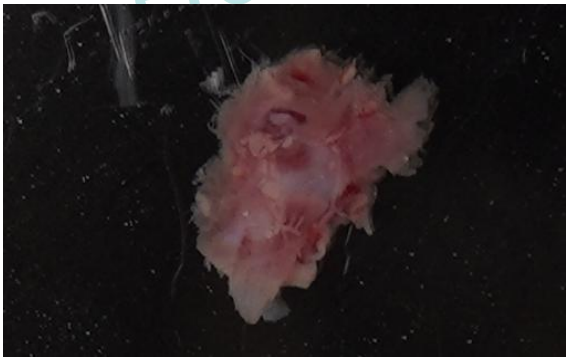

Figure 2a. Excised acetabular cartilage tissue

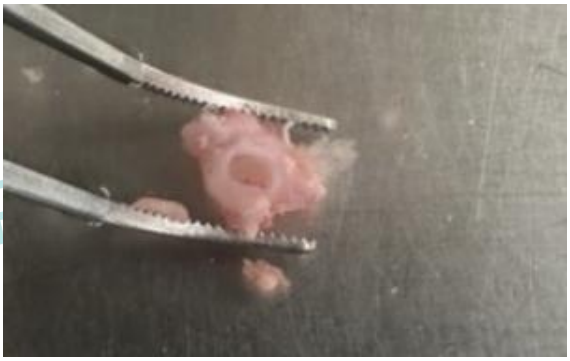

Figure 2b. Excised acetabular cartilage tissue

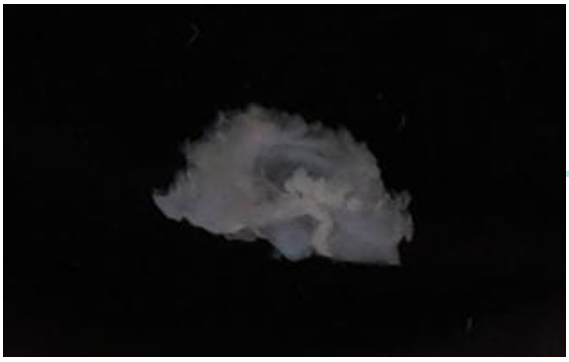

Figure 3a. Cleaned acetabular cartilage tissue

Figure 3b. Cleaned acetabular cartilage tissue