# Rat Temporomandibular Joint Chondrocyte Isolation and Culture Kit

**Cat. No.: P-CA-625**

**Size: 3Tests / 10Tests**

## Background

The Rat Temporomandibular Joint Chondrocyte Isolation and Culture Kit is specifically developed for the extraction of primary Rat Temporomandibular Joint Chondrocytes. 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 $1\times10^6$ cells. When subcultured at a 1:2 ratio, the cells can undergo 3-5 passages, with the optimal 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 Temporomandibular Joint Chondrocytes from various rat strains (e.g., Wistar, SD) aged 14 days. After processes of tissue isolation, enzymatic digestion, and 48-hour plating, a yield of $>1\times10^6$ cells can be obtained.

**Note: 16 temporomandibular joint cartilage tissues extracted from 8 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 the temporomandibular joint cartilage 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 Temporomandibular Joint Chondrocytes | 3Tests (250 mL)<br>10Tests (500 mL×2) | Pale Yellow Clear Liquid | 2-8°C, 1 year |
| Specialized Digestive Solution for Rat Temporomandibular Joint Chondrocytes | 3Tests (15 mL)<br>10Tests (50 mL) | Yellow Clear Liquid | -5~-20°C, 1 year |
| Basic Culture Medium for Rat Temporomandibular Joint Chondrocytes | 3Tests (50 mL)<br>10Tests (100 mL) | Red Clear Liquid | 2-8°C, 1 year |
| Supplement for Rat Temporomandibular Joint Chondrocytes | 3Tests (10 mL)<br>10Tests (20 mL) | Yellow Clear Liquid | -5~-20°C, 1 year |
| 70 μm Cell Filter | 3Tests (3 pcs)<br>10Tests (10 pcs) | N/A | Room temperature, 3 years |
| 100 μm Cell Filter | 3Tests (3 pcs)<br>10Tests (10 pcs) | N/A | Room temperature, 3 years |

**Note:**

1. **All components should be stored according to the temperature indicated on the labels of the reagent tubes.**
2. **The reagents stored at -5~-20℃ (such as Specialized Digestive Solution for Rat Temporomandibular Joint 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 rats to familiarize yourself with the 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 (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 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 Temporomandibular Joint Chondrocytes & Supplement for Rat Temporomandibular Joint Chondrocytes: thaw at 4°C and equilibrate to room temperature.
   ② Specialized Washing Solution for Rat Temporomandibular Joint Chondrocytes & Basic Culture Medium for Rat Temporomandibular Joint Chondrocytes: equilibrate to room temperature.
3) Preparation of Complete Culture Medium: Add 10 mL of Supplement for Rat Temporomandibular Joint Chondrocytes into 50 mL of Basic Culture Medium for Rat Temporomandibular Joint 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 for Rat Temporomandibular Joint Chondrocytes 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, Ophthalmic Scissors 3 and Curved Forceps 3).

   **Note: The distal third of the instruments should extend beyond the EP tube racks 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 prone position within the clean bench using needles, preparing for tissue harvesting.
   ③ Tissue Harvesting Procedure:
      a. Use straight forceps 1 to firmly clamp the skin at the rat's neck, employ ophthalmic scissors 1 to cut through all skin and ear tissue from the neck to the nasal region, thereby completely exposing the skull.

         **Note: Maximize skin removal while leaving minimal fur around the snout. Vigorously tear residual hair away from the dissection area to prevent contamination.**
      b. Use straight forceps 1 to grasp the rat's mouth vertically for fixation. Separate the muscle layers adjacent to the mandible with ophthalmic scissors 2, exposing the mandible, and locate the temporomandibular joint and condyle.

**Note: Perform blunt dissection of the muscle layers using scissors or forceps to avoid cutting the mandible. If the temporomandibular joint is not immediately identifiable, gently manipulate the rat's lower jaw with straight forceps; the junction between the mandible and temporal bone (i.e., the cheek area) corresponds to the temporomandibular joint.**

c. Using straight forceps 1 to vertically fix the rat's mouth, gently insert ophthalmic scissors 2 into the junction between the mandible and temporal bone. Gently lift the ophthalmic scissors to separate the mandible from the temporal bone, exposing the condyle and the white cartilage tissue at its anterior end. Use ophthalmic scissors 2 to excise the condyle, and transfer it to a culture dish containing 10 mL of Specialized Washing Solution for Rat Temporomandibular Joint Chondrocytes using curved forceps 3.

**Note: During the entire sampling process, only the first set of scissors and forceps can come into contact with the external skin of the rats. All other instruments must not touch the external skin or hair. If contact occurs, sterile instruments must be replaced to prevent contamination.**

## 3. Tissue Processing and Digestion

1) Tissue Processing

① Put Micro Straight Forceps ,micro curved forceps, micro scissors on the EP tube rack within the clean bench, ensuring the distal third of each tool suspended, spray 75% medical alcohol onto the dissecting microscope, then place it inside the clean bench..

② Using this new set of micro forceps, manipulate the tissue under a dissection microscope: Stabilize the tissue with micro straight forceps in the left hand, and surgically dissect the lateral tunica albuginea with micro curved forceps in the right hand. Harvest the pure cartilage tissue from the apex, then separate the cartilage tissue from the condyle using micro scissors, retaining the purified cartilage tissue.

③ Stabilize the cartilage tissue with micro straight forceps in the left hand, and bisect the processed cartilage tissue into two equal fragments using ophthalmic scissors in the right hand.

2) Tissue Digestion

① Add 5 mL of Specialized Digestive Solution for Rat Temporomandibular Joint 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 Temporomandibular Joint 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 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 Rat Temporomandibular Joint 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 Temporomandibular Joint 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 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 Temporomandibular Joint 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 Temporomandibular Joint Chondrocytes, then inoculat into the T25 cell culture flask. The cells were cultured in a incubator at 37°C, 5% $CO_2$. After 2 to 3 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 Temporomandibular Joint 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 outer membrane is completely removed |
| 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

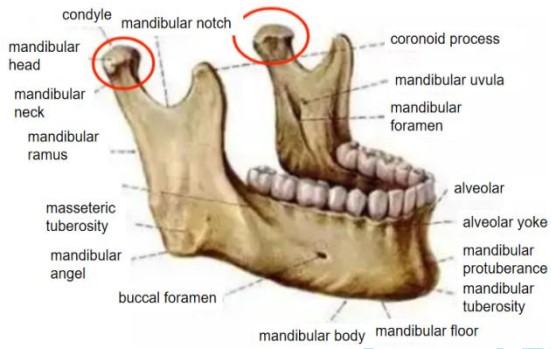


**Figure 1a. Diagram of Human Temporomandibular Anatomy**

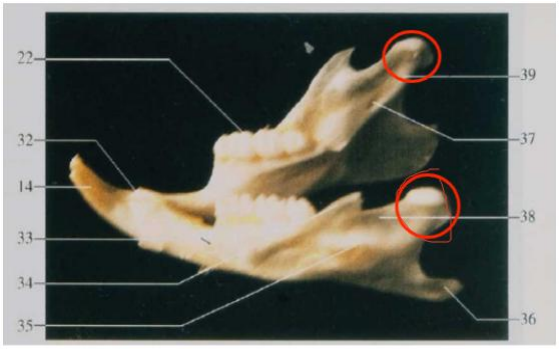


**Figure 1b. Diagram of Mouse Temporomandibular Anatomy**

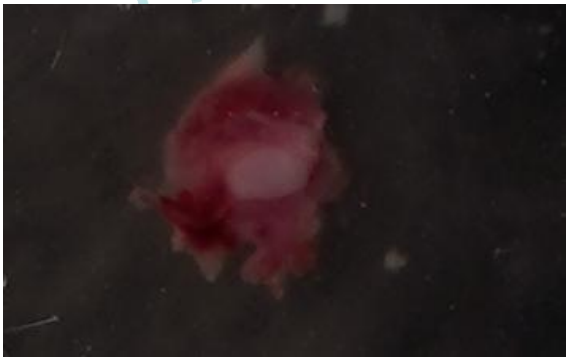

**Figure 2a. Excised temporomandibular joint tissue tissue**

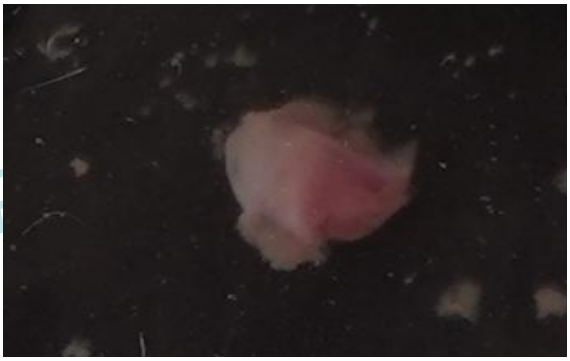

**Figure 2b. Excised temporomandibular joint tissue tissue**



**Figure 3. Cleaned temporomandibular joint tissue**