Rat Intervertebral Disc Annulus Fibrosus Cell Isolation and Culture Kit

Cat. No.: P-CA-626

Size: 3Tests / 10Tests

Background

The Rat Intervertebral Disc Annulus Fibrosus Cell Isolation and Culture Kit is specifically developed for the extraction of primary Rat Intervertebral Disc Annulus Fibrosus Cells. 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. 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 I positive rate) has been confirmed to exceed 90%.

Scope of Application

This product is suitable for extracting Rat Intervertebral Disc Annulus Fibrosus Cells 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: Intervertebral Disc Annulus Fibrosus 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 annulus fibrosus tissue of intervertebral disc 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 Intervertebral Disc Annulus Fibrosus Cells	3Tests (250 mL) 10Tests (500 mL×2)	Pale Yellow Clear Liquid	2-8°C, 1 year
Specialized Digestive Solution for Rat Intervertebral Disc Annulus Fibrosus Cells	3Tests (15 mL) 10Tests (50 mL)	Yellow Clear Liquid	-5~-20°C, 1 year
Basic Culture Medium for Rat Intervertebral Disc Annulus Fibrosus Cells	3Tests (50 mL) 10Tests (100 mL)	Red Clear Liquid	2-8°C, 1 year
Supplement for Rat Intervertebral Disc Annulus Fibrosus Cells	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 Rat Intervertebral Disc Annulus Fibrosus 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°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 (At least 3 pairs of ophthalmic scissors, 1 pair of straight forceps, 2 pairs of curved forceps, 1 pair of scalpel, 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 Intervertebral Disc Annulus Fibrosus Cells & Supplement for Rat Intervertebral Disc Annulus Fibrosus Cells: Thaw at 4°C and equilibrate to room temperature.
   ② Specialized Washing Solution for Rat Intervertebral Disc Annulus Fibrosus Cells & Basic Culture Medium for Rat Intervertebral Disc Annulus Fibrosus Cells: Equilibrate to room temperature.
3) Preparation of Complete Culture Medium: Add 10 mL of Supplement for Rat Intervertebral Disc Annulus Fibrosus Cells into 50 mL of Basic Culture Medium for Rat Intervertebral Disc Annulus Fibrosus Cells, 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, 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-contamination.
   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 grasp the dorsal skin of the rat. With ophthalmic scissors 1, incise the lifted skin along the midline to expose the underlying dorsal musculature.

      Note: Ensure complete exposure from the cervical to sacral region. Retract fur away from the dissection field to prevent contamination.
      b. With the left hand using curved forceps 2 for stabilization, use ophthalmic scissors 2 in the right hand to resect excess cervical adipose tissue , sever the cervical vertebraeand bilateral transection of ribsIsolate the spinal column.
      c. Stabilize the spine with curved forceps 2 in the left hand. Using curved forceps 3 and

ophthalmic scissors 2 in the right hand, meticulously dissect residual muscles and connective tissue from the vertebral surface.

d. Stabilize the spine with curved forceps 2. Insert one blade of ophthalmic scissors 2 along the spinal canal. Bilaterally transect the connections between disc tissue and vertebral bone at the whitish intervertebral junctions to isolate intervertebral disc tissue.

e. Using curved forceps 2 in the left hand to assist in fixing the spine, and the right hand holds the scalpel, excise the whitish annulus fibrosus tissue bilaterally. Transfer disc tissue using curved forceps 3 to a new Petri dish containing 10 mL of rat-specific annulus fibrosus cell washing solution.

Note: Healthy IVD tissue appears whitish-translucent with elastic properties. If hard bony structures are encountered (indicating vertebral bone), withdraw the blade and reorient along the red-white vertebral-disc junction.

3. Tissue Processing and Digestion

1) Tissue Processing

① Hold curved forceps 3 in the left hand and a scalpel in the right hand to manipulate the annulus fibrosus tissue of the intervertebral disc: Rinse the harvested disc tissue once to remove excess blood clots and connective tissue. Transfer the tissue to a new culture dish containing 10 mL of rat annulus fibrosus cell-specific cleaning solution.

② Using curved forceps 3 in the left hand to stabilize the tissue, excise excess fibrous tissue surrounding the disc with the scalpel in the right hand. Use the scalpel or micro forceps to extract the nucleus pulposus from the central disc region, creating an incision at the peripheral annulus fibrosus.

2) Tissue Digestion

① Add 5 mL of Specialized Digestive Solution for Rat Intervertebral Disc Annulus Fibrosus Cells 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 Intervertebral Disc Annulus Fibrosus Cells.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. (Standard: No obvious tissue blocks)

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 Intervertebral Disc Annulus Fibrosus Cells. 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 Intervertebral Disc Annulus Fibrosus Cells 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 Intervertebral Disc Annulus Fibrosus Cells 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 Intervertebral Disc Annulus Fibrosus Cells, 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 Intervertebral Disc Annulus Fibrosus Cells 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 tissues are inpure	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

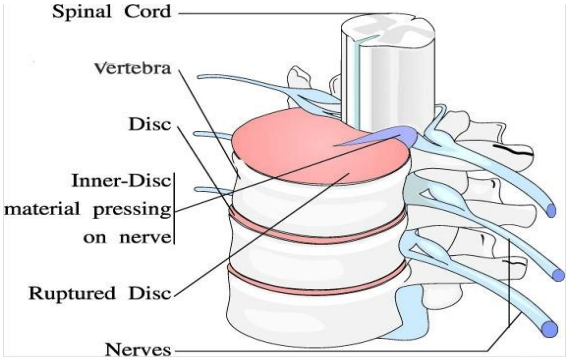


Figure 1a. Intervertebral Disc and Annulus Fibrosus Schematic

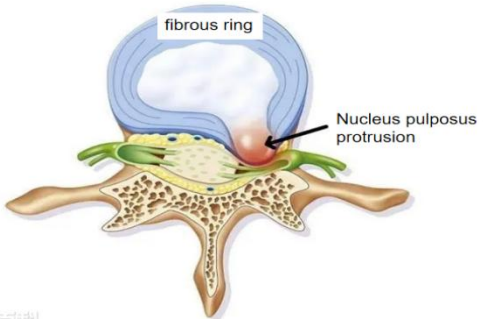


Figure 1b. Intervertebral Disc and Annulus Fibrosus Schematic



Figure 2. Excised spinal tissue



Figure 3. Remove excess blood vessels and muscles from the spine

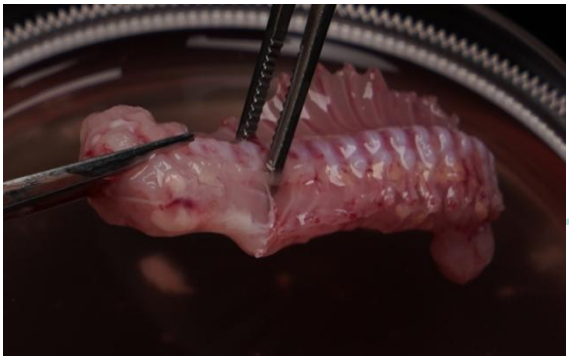


Figure 4a. Cut off excess ribs on both sides of the spine and preserve pure spinal tissue

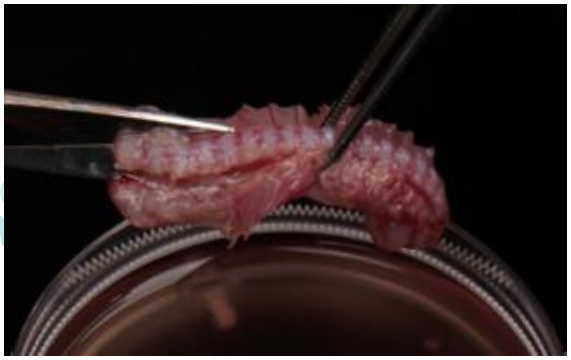


Figure 4b.Cut off excess ribs on both sides of the spine and preserve pure spinal tissue



Figure 5. Clean spinal tissue



Figure 6a. Cut off the bones on both sides of the white intervertebral disc, leaving the intervertebral disc intact

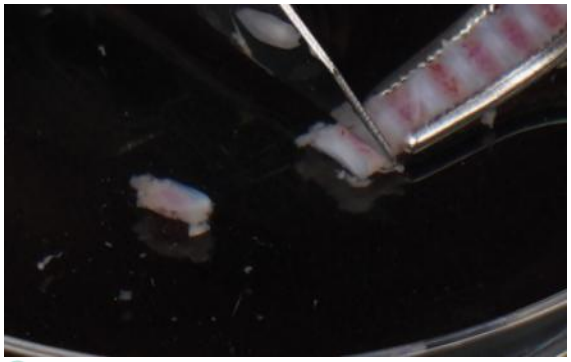


Figure 6b. Cut off the bones on both sides of the white intervertebral disc, leaving the intervertebral disc intact



Figure 7. Intervertebral disc tissue

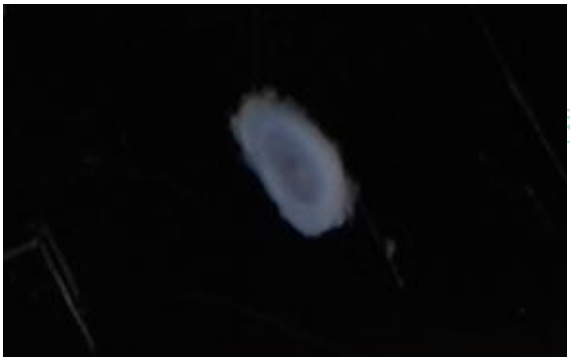


Figure 8. Clean up excess muscle and connective tissue on the outer side of the intervertebral disc tissue



Figure 9. Pull out the middle nucleus pulposus tissue



Figure 10. Chop and digest

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