# Rabbit Intervertebral Disc Annulus Fibrosus Cells

**Cat. No. : CP-Rb032**

## General Information

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| **Species** | Rabbit/New Zealand White rabbit |
| **Tissue Type** | Skeletal system |
| **Tissue** | Intervertebral disc tissue |
| **Cell Type** | Other cell |
| **Morphology** | Spindle-shaped; polygonal |
| **Growth Properties** | Adherent |
| **Storage Conditions** | For long-term cryopreservation, cryovials should be stored in liquid nitrogen at −150°C to −196°C. Storage at −80°C is restricted to short-term interim use only. |

## Culture Conditions and Handling

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| **Complete Medium** | Rabbit Intervertebral Disc Annulus Fibrosus Cell Complete Medium[CM-Rb032] |
| **Population Doublings** | Guaranteed to further expand for 10 population doublings |
| **Subcultivation Ratio** | 1:2 |
| **Dissociation Reagent** | 0.25% trypsin |
| **Medium Renewal** | Every 2 to 3 days |
| **Freezing Medium** | General Freezing Medium[PB180436] |
| **Incubation Atmosphere** | Air, 95%; $CO_2$, 5% |
| **Temperature** | 37°C |
| **Instructions** | 1. Check all containers for leakage or breakage.<br>2. Remove the frozen cells from the dry ice packaging and immediately transfer them to liquid nitrogen (liquid or vapor phase) for long-term cryopreservation. |
| **Subculturing Procedure** | Adherent cell dissociation<br>1. Aspirate the culture medium from the T25 flask and wash the cells once with phosphate-buffered saline (PBS).<br>2. Add 1 mL of 0.25% trypsin solution to the T25 flask. Gently swirl the flask to ensure the solution fully covers the bottom surface, then aspirate 500–700 µL of the trypsin solution. Incubate at 37°C for 1–3 min. Examine the cells under an inverted microscope. Once cells become rounded and retracted, add 5 mL of complete growth medium to neutralize the digestion.<br>3. Gently pipette to mix and dissociate the cells. Subculture into a new T25 flask at the recommended split ratio, and top up with fresh complete growth medium to a final volume of 5 mL. Incubate the flask in a humidified incubator at 37°C with 5% $CO_2$ .<br>4. Once the cells have fully adhered, assess cell viability and confluency under a microscope prior to downstream experiments. Subsequently, refresh the medium with fresh complete growth medium per the standard cell maintenance protocol. |

## Background

Rabbit intervertebral disc annulus fibrosus cells (*Oryctolagus cuniculus*) are isolated from intervertebral disc tissue. They are the predominant cell type that constitutes the intervertebral disc annulus fibrosus. These spindle-shaped cells are arranged in a concentric circular pattern and express type I collagen and type II collagen. Their core functions include: synthesizing and secreting annulus fibrosus matrix to maintain the structural integrity of intervertebral discs; restricting the outward protrusion of the nucleus pulposus; and bearing and transmitting the torsional and tensile loads of the spine. Degeneration and apoptosis of rabbit intervertebral disc annulus fibrosus cells are closely associated with intervertebral disc degeneration and lumbar disc herniation. In vitro-cultured rabbit intervertebral disc annulus fibrosus cells serve as an important cell model for investigating spinal diseases.

Rabbit intervertebral disc annulus fibrosus cells isolated from Procell Laboratory are prepared by combined collagenase-dispase digestion, followed by selective culture in specialized chondrocyte medium. Each vial contains approximately $5 \times 10^5$ cells.

Rabbit intervertebral disc annulus fibrosus cells isolated from Procell Laboratory exhibit positive immunofluorescence staining for type I collagen, with a purity greater than 90%. In addition, the cells test negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast, and fungi.

## Handling Recommendations for Cryopreserved Cells

### Preparations Before Thawing

1. Primary cells have high nutritional demands; therefore, prepare complete culture medium in advance.
2. Preheat the complete culture medium to 37°C for 30 minutes.
3. Prepare 9 mL of complete medium within a sterile centrifuge tube to dilute the cryoprotectant present in the frozen cell suspension.
4. Use sterile gloves to protect the cryovial from contact with the water bath water, and preventing cell contamination.

### Thawing Procedure

1. Retrieve the cryovial containing frozen cells from liquid nitrogen storage. Place it in sterile gloves and immediately submerge it in a 37°C water bath.
2. Thaw the cells rapidly (<1 minute) by gently swirling the vial in the 37°C water bath until only a small ice crystal remains.
3. Transfer the vial to a laminar flow hood. Wipe the exterior of the vial with 75% ethanol before opening.
4. Transfer the thawed cells dropwise into a preheated 9 mL centrifuge tube containing complete medium.
5. Centrifuge the cell suspension at approximately 250 × g for 5 minutes. For cells characterized by low density and small volume, such as lymphocytes and suspended cells, the centrifugation speed can be appropriately increased to 400 × g for 8 minutes.
6. After centrifugation, discard the supernatant and retain the cell pellet.
7. Gently resuspend the cells in complete growth medium, then transfer them to an appropriate culture vessel and place it in the recommended culture environment.

### Notes

1. The entire recovery process should be completed as quickly as possible.
2. Select the culture vessel size based on the number of cells in the cryovial. Procell single cryovials are recommended for resuspension in 6 cm dishes or T25 flasks.
3. Minimize the time thawed cells are kept at room temperature. DMSO must be immediately diluted or removed by centrifugation.

## Precautions After Receipt of Frozen Cells

1. Upon unpacking, inspect the condition of the frozen cells and dry ice, and take photographs immediately. The following after-sales service will be provided based on these photographs, including assessment of the remaining dry ice, verification that the cryovial was fully buried in dry ice, and evaluation of whether the cells thawed and refrozen during transit.
2. Upon receipt, the cells should be transferred to liquid nitrogen immediately or directly resuscitated. If liquid nitrogen is unavailable, the cells can be temporarily stored at -80°C, however, the storage period should be limited to less than one week whenever possible. Prolonged storage at -80°C may gradually reduce post-thaw cell viability, and the extent of this viability loss is unpredictable.
3. Ensure that the operator has sufficient knowledge and experience in cell culture, and the laboratory is equipped with essential instruments, including a biosafety cabinet, $CO_2$ incubator, inverted microscope, centrifuge, water bath. Carefully review the cell instruction manual to understand key cell characteristics including growth properties (adherent or suspension), morphology, basal medium requirements, serum concentration, cytokine supplementation, subcultivation ratio, medium renewal schedule.
4. After resuscitation, observe the cells under a microscope and record the cell status by taking photographs (1-3 images each at 100× and 200× magnification for 3 consecutive days). These images will serve as supporting documentation for follow-up services. In addition, a small aliquot of cells may be used to assess cell viability by automated cell counting or trypan blue staining.

**Note:** Cells should not be observed too frequently within the first 24 hours after resuscitation, as this may affect cell growth or adherence. Observation once per day is sufficient.

5. Primary cells have a limited number of passages; it is recommended that they be used as soon as possible.

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