cyagen | OriCell™

User Manual

OriCell™ Rabbit Adipose-derived Mesenchymal Stem Cells With RFP

Catalog No. RBXMD-01201

We help you discover life

Introduction

Adipose-derived mesenchymal stem cells are a type of pluripotent stem cells that exist in adipose matrix. Because of its strong proliferation ability and immune regulation function, it is widely used in the fields of tissue engineering, cell therapy and gene therapy.

As a research hotspot, rabbit adipose-derived mesenchymal stem cells are widely used in regenerative medicine and tissue engineering, especially in the fields of bone, cardiovascular and nervous system diseases.

OriCell™ Rabbit Adipose-derived Mesenchymal Stem Cells With RFP were taken from the inguinal fat of New Zealand white rabbits. These cells can express specific proteins of ADSCs and have strong proliferation and multidirectional differentiation capabilities. It can be used as a cell model to study proliferation, aging, immunity, differentiation and transplantation.

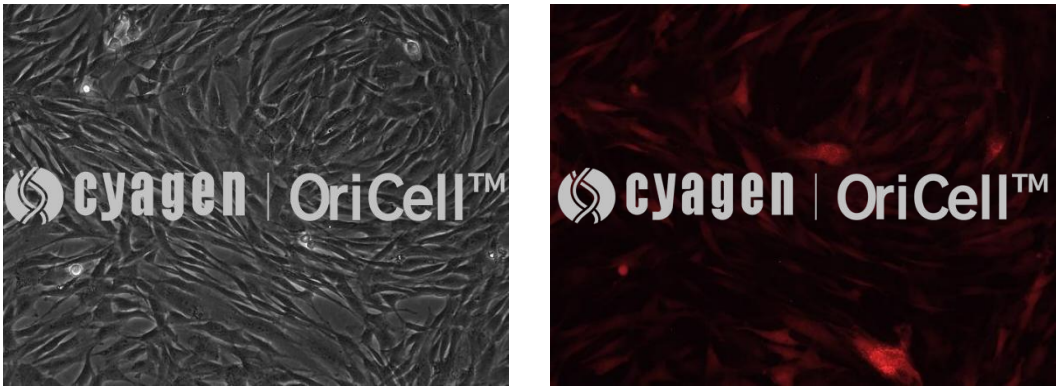
Note: **This product is only provided for further scientific research. It is not intended for diagnostic, therapeutic, clinical, household, or any other applications.**

When citing our products in academic journals, please indicate "OriCell™ + Catalog Number, from Cyagen Biosciences (Guangzhou) Inc."

Product Information

Name	OriCell™ Rabbit Adipose-derived Mesenchymal Stem Cells With RFP
Catalog Number	RBXMD-01201
Number of Cells	1×10^6 cells/vial
Passage Number	P2
Storage at	Liquid Nitrogen (-196°C)

The Shape of OriCell™ Rabbit Adipose-derived Mesenchymal Stem Cells With RFP



QC

- Pass the detection of bacteria, fungi, mycoplasma, and endotoxins.
- Pass the viability examination. The viable rates is higher than 80%.
- The cell doubling time is less than 72 hours.
- Flow cytometry showed that Integrin beta 1 and CD44 are positive (>70%), while C-Kit, VCAM1, CD31 and CD34 are negative (<5%).
- The cells can be induced to differentiate into osteoblasts, adipocytes, chondrocytes, etc.

Please reference "COA" for details.

General Handing Principles

1. Ensure that all equipment is kept clean and tidy.
2. Please follow the instructions.
3. Use suitable and reliable consumables and reagents.
4. Adipose-derived mesenchymal stem cells have limited ability to proliferate in vitro and cannot maintain their differentiation potential for a long time. OriCell™ Rabbit Adipose-derived

Mesenchymal Stem Cells With RFP can be passaged for more than 5 times and still maintain all indicators qualified. But we always recommend using lower generation cells for scientific research.

5. Usually the inoculation density of rabbit adipose-derived mesenchymal stem cells is (2.5~4) $\times 10^4$ live cells/cm^2.

Note: **The cryopreservation solution of this product contains DMSO, which has potential risks. Please handle it carefully.**

Thawing and Culturing of Cells

Materials Required

- OriCell™ Rabbit Adipose-derived Mesenchymal Stem Cells With RFP (Cat. No. RBXMD-01201)
- OriCell™ Complete Medium For Rabbit Adipose-derived Mesenchymal Stem Cells (Cat. No. RBXMD-90011)

Steps

Note: **If the received cells are thawed within 24 hours, they can be stored in a refrigerator at -80°C. If more than 24 hours, please store them in liquid nitrogen. Please take them out 10 minutes early before thawing and place them at -80°C to allow the liquid nitrogen in the tube to evaporate.**

1. Preheat the water bath at 37°C.
2. Warm the complete medium to 37°C.
3. Add more than 5 mL of complete medium to a 15 mL centrifuge tube for use.
4. Take the cells out of the -80°C refrigerator, put them in a 37°C water bath and shake them quickly to thaw the cryopreservation solution.

   Note: **During the thawing process, the cryotube must be shaken to ensure that the solution thaws quickly and evenly.**
5. When shaking, please avoid water immersing the pipe cover to cause pollution.

6. When the cryopreservation solution has thawed into ice crystal with a diameter of about 2 mm, stop the water bath. Continue to shake the cryotube until the ice crystal melts thoroughly.
7. Wipe the outer surface of the cryotube with 75% ethanol.
8. Open the cryopreservation tube in the ultraclean bench, use a Pasteur pipette to suck the cell suspension, and transfer it to the prepared centrifuge tube.
9. Wash the cryotube once with 1 mL of complete medium to collect residual cells to reduce loss.
10. Centrifuge the cell suspension at 250×g for 4 minutes.
11. Remove the supernatant after centrifugation. Add 2 mL of complete medium, gently pipette the cell pellet, blow and mix thoroughly.
12. Inoculate the cells into a T25 flask or a culture container with an equivalent bottom area. Add enough complete medium, the total amount of medium in a T25 flask should not less than 5 mL.
13. Shake the cells well and incubate them in a CO_2 incubator at saturated humidity, 37°C, 5% CO_2 inside.

    Note: **Do not move or observe the cells within 2 hours of inoculation. This will seriously affect cell adhesion, resulting in poor shape, cell clumping, and uneven adhesion.**
14. On the next day of recovery, observe the cell status, and replace medium with fresh complete medium or passage.

    Note: **If you find lots of floating cells or other abnormal conditions, please investigate the cause in time and contact us.**
15. Then refresh the complete medium every 2 days until the cells have grown to 90% confluence, which requires passage generation.

Passaging of Cells

Materials Required

- OriCell™ 0.25% Trypsin-0.04% EDTA Solution (Cat. No. TEDTA-10001)
- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No. PBS-10001)
- OriCell™ Complete Medium For Rabbit Adipose-derived Mesenchymal Stem Cells (Cat. No. RBXMD-90011)

Steps

1. Prewarm the complete medium and trypsin to 37°C.
2. Remove the medium in the culture container.
3. Wash the cells twice with PBS (approximately 3 mL for T25 flask and 6 mL for T75 flask). Please perform relatively slightly and wash thoroughly. Remove the PBS.
4. Add trypsin (approximately 1.5 mL for T25 flask and 3 mL for T75 flask), spread quickly to ensure full contact with the cells.
5. Observe the cells under a microscope. After about 70%~80% of the cells have shrunk and round, tap the outer wall of the culture vessel to remove the cells from the culture surface.
6. Add complete medium (approximately 3 mL for T25 flask and 6 mL for T75 flask) immediately, and then slightly shake the culture container to mix the medium and trypsin quickly to stop the digestion.
7. Use a pipette to suck up the cell suspension, pipetting the bottom surface of the culture container several times, and pipetting down as much as possible of the cells.

   Note: **The pipetting action should not be violent.**
8. Transfer the cell suspension to a centrifuge tube. Wash the container once with PBS (approximately 3 mL for T25 flask and 6 mL for T75 flask) to collect residual cells.
9. All the collected cell suspensions are centrifuged at 250×g for 4 minutes.

10. Remove the supernatant after centrifugation. Add 2 mL of complete medium, gently pipette the cell pellet, blow and mix thoroughly.
11. Inoculate the cells into a suitable culture container at (2.5~4)×10^4 live cells/cm^2, or adjust the passage ratio according to the actual growth of the cells.

    Note: **OriCell™ Rabbit Adipose-derived Mesenchymal Stem Cells usually have a passage ratio of 1:3, and they will grow to reach confluence within 72 hours.**
12. Shake the cells well and incubate them in a CO_2 incubator at saturated humidity, 37°C, 5% CO_2 inside.
13. Then refresh the complete medium every 2 days until the cells have grown to 90% confluence, which requires passage generation or frozen.

    Note: **Under normal conditions, the growth time of rabbit adipose-derived mesenchymal stem cells does not exceed 72 hours per generation, and there is no need to change the medium. Frequent fluid changes will destroy the built-up cellular micro-environment.**

Cryopreservation of Cells

Materials Required

- OriCell™ NCR Protein-Free Cryopreservation Medium For General Use (Cat. No. NCPF-10001)
- OriCell™ NCR Cryopreservation Medium For General Use (Cat. No. NCRC-10001)

Steps

1. The cells are cryopreserved after growing to appropriate density that can be passaged.
2. For cell digestion, please refer to OriCell™ Rabbit Adipose-derived Mesenchymal Stem Cells With RFP "Passaging Steps 1~9".
3. The cells are uniformly suspended with an appropriate amount of cryopreserved solution, then the supernatant is removed after centrifugation.
4. The cells are divided into cryopreservation tubes based on proportion or quantity.
5. If you choose OriCell™ NCR Cryopreservation Medium, please disperse the cryopreservation tube directly into the refrigerator at -80°C.

Note: During the cryopreservation of cells, especially within 4 hours of the beginning, the refrigerator door should not be opened, which will seriously affect the survival rate of cells.

6. After 8 hours, cells can be transferred to liquid nitrogen for long-term storage.
   Note: We suggest that the storage time in the refrigerator at -80°C should not exceed 48 hours.