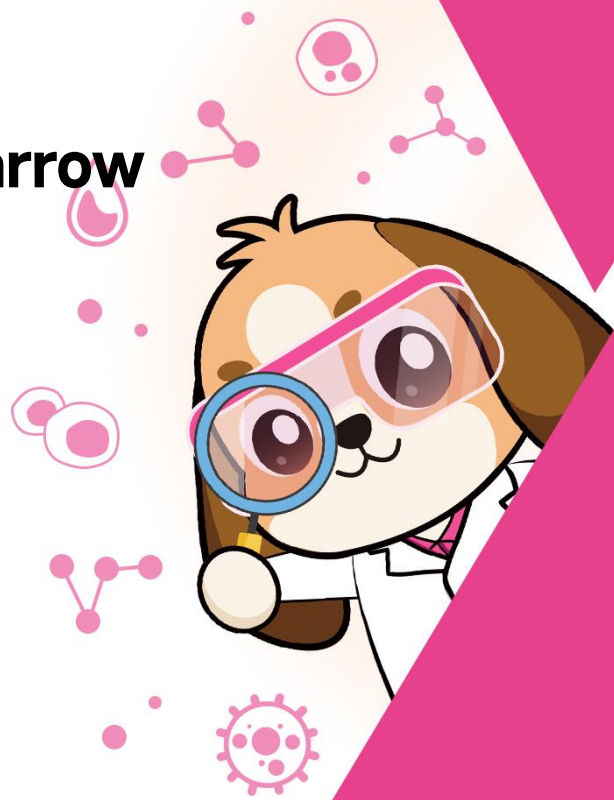


User Manual

OriCell™ F344 Rat Bone Marrow Mesenchymal Stem Cells With GFP

Catalog No. RAFMX-01101



Introduction

Bone marrow mesenchymal stem cells (BMSCs) are a type of multipotent stem cell residing in the bone marrow stroma. Due to their strong proliferative capacity and immunomodulatory properties, BMSCs are widely applied in tissue engineering, cell therapy, and gene therapy.

As a research hotspot, F344 rat bone marrow mesenchymal stem cells are commonly used in regenerative medicine and tissue engineering, particularly in studies involving bone, cardiovascular, and nervous system disorders.

OriCell™ F344 Rat Bone Marrow Mesenchymal Stem Cells are isolated from the bone marrow of healthy F344 rats. These cells exhibit robust proliferation and multipotent differentiation potential. Following adherent culture, they can be stably transduced with a GFP-expressing lentiviral vector to achieve persistent GFP expression. They serve as a versatile cell model for investigating proliferation, aging, immune regulation, differentiation, and transplantation.

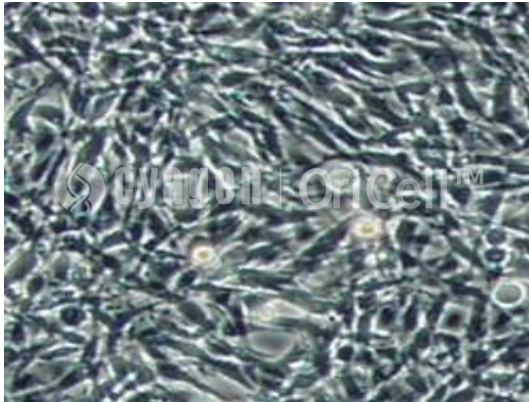
Note: This product is only provided for further scientific research. It is not intended for diagnostic, therapeutic, clinical, household, or any other applications.

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

Product Information

Name	OriCell™ F344 Rat Bone Marrow Mesenchymal Stem Cells With GFP
Catalog Number	RAFMX-01101
Amount of Cells	1×10 ⁶ cells/vial
Passage Number	P5
Storage at	Liquid Nitrogen (-196°C)

The Morphology of OriCell™ F344 Rat Bone Marrow Mesenchymal Stem Cells With GFP



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 CD29, CD44, CD90 are positive (>70%), while CD34, CD45, CD11b are negative (<5%).
- The cells can be induced to differentiate into osteoblasts, adipocytes, chondrocytes, etc.

Please refer to "COA" for details.

General Handling 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. Bone marrow mesenchymal stem cells have limited ability to proliferate in vitro and cannot maintain their differentiation potential for a long time. OriCell™ F344 Rat Bone Marrow Mesenchymal Stem Cells With GFP 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.

- Usually the inoculation density of rat bone marrow mesenchymal stem cells is $(2.5-4) \times 10^4$ live cells/cm².

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

Thawing and Establishing of Cells

Materials Required

- OriCell™ F344 Rat Bone Marrow Mesenchymal Stem Cells With GFP (Cat. No.: RAFMX-01101)
- OriCell™ Complete Medium For Rat Bone Marrow Mesenchymal Stem Cells (Cat. No.: RAXMX-90011)

Steps

Note: If the received cells are to be thawed within 24 hours, they can be stored in an ultra-low temperature freezer at -80°C. For storage longer than 24 hours, please keep them in liquid nitrogen. Before thawing, transfer the cells from liquid nitrogen to -80°C and hold them there for 10 minutes to allow any residual 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 at least 5 mL of complete medium to a 15 mL centrifuge tube for subsequent use.
4. Remove the cryovial containing cells from the -80°C freezer, immerse it in the 37°C water bath, and gently and quickly swirl to thaw the cryopreservation solution.

Note:

(1) Gently shake the cryovial during thawing to ensure rapid and uniform thawing.

(2) Avoid submerging the cap in water to prevent contamination.

(3) Stop thawing in the water bath when only a single ice crystal (approximately 2 mm in diameter) remains, then continue gently shaking the vial until it fully melts.

5. Wipe the outer surface of the cryovial with 75% ethanol.
6. In a biosafety cabinet, open the cryovial and transfer the cell suspension to the prepared centrifuge tube using a Pasteur pipette.
7. Rinse the cryovial once with 1 mL of complete medium to collect residual cells and minimize loss.
8. Centrifuge the cell suspension at 250×g for 4 minutes.
9. Carefully remove the supernatant after centrifugation. Add 2 mL of complete medium, gently resuspend the cell pellet by pipetting up and down to mix thoroughly.
10. Inoculate the cells into a T25 flask or culture vessel with an equivalent growth surface area. Add sufficient complete medium so that the total volume in a T25 flask is no less than 5 mL.
11. Gently swirl the flask to evenly distribute the cells, then incubate in a CO₂ incubator at 37°C with 5% CO₂ and saturated humidity.

Note: Do not disturb or observe the cells within the first 2 hours after seeding, as this may affect cell adhesion, resulting in poor morphology, clumping, or uneven attachment.

12. The day after recovery, observe the cell status and either replace the medium with fresh complete medium or passage the cells as necessary.

Note: If an excessive number of floating cells or any abnormal conditions are observed, investigate promptly and contact us for assistance.

13. Refresh the complete medium every 2 days until the cells reach approximately 90% confluence, at which point passage is required.

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 Rat Bone Marrow Mesenchymal Stem Cells (Cat. No.: RAXMX-90011)

Steps

1. Prewarm the complete medium and trypsin to 37°C.
2. Transfer the medium from the culture vessel to a centrifuge tube.
3. Gently wash the cells twice with PBS (approximately 3 mL for a T25 flask and 6 mL for a T75 flask). Ensure thorough washing but avoid excessive force. Then remove the PBS.
4. Add trypsin (approximately 1.5 mL for a T25 flask and 3 mL for a T75 flask), quickly spread it to ensure full coverage of the cell layer.
5. Observe the cells under a microscope. When approximately 70% to 80% of the cells have shrunk and become round, gently tap the outer wall of the culture vessel to detach the cells from the surface.
6. Immediately add complete medium (approximately 3 mL for a T25 flask and 6 mL for a T75 flask), then gently swirl the culture vessel to mix the medium and trypsin, stopping the digestion process.
7. Collect the cell suspension using a pipette, gently pipetting along the bottom of the vessel several times to ensure maximal recovery of the cells.

Note: Pipetting should be performed gently to avoid damaging the cells.

8. Transfer the cell suspension to a centrifuge tube. Rinse the culture vessel once with PBS (approximately 3 mL for a T25 flask and 6 mL for a T75 flask) to collect any remaining cells



and add the wash to the centrifuge tube.

9. Centrifuge all collected cell suspensions at $250 \times g$ for 4 minutes.
10. Carefully remove the supernatant after centrifugation. Add 2 mL of complete medium and gently resuspend the cell pellet by pipetting up and down to thoroughly mix.
11. Inoculate the cells into a suitable culture vessel at a density of $(2.5-4) \times 10^4$ live cells/cm², or adjust the seeding density based on the actual growth conditions of the cells.

Note: OriCell™ F344 Rat Bone Marrow Mesenchymal Stem Cells With GFP usually have a passage ratio of 1:3, and they will grow to reach confluence within 72 hours.

12. Shake the cells gently and place them in an incubator at 37 °C, 5% CO₂, and saturated humidity.
13. Refresh the culture medium every 2 days. When cell confluence exceeds 90%, passage or cryopreserve the cells.

Note: Under normal conditions, the growth time of F344 Rat Bone Marrow Mesenchymal Stem Cells With GFP 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. Cells should be cryopreserved once they reach an appropriate density suitable for passaging.
2. For cell digestion, please refer to passaging steps 1-9 above.
3. Carefully remove the supernatant after centrifugation and gently resuspend the cells in an appropriate volume of cryopreservation medium.
4. Aliquot the cells into cryovials according to the desired cell number or proportion.
5. When using any of the recommended NCR cryopreservation media above, cryovials can be directly placed individually into a -80°C freezer.

Note: Avoid opening the freezer door during the first 4 hours of freezing, as temperature fluctuations can seriously impact cell viability.

6. After approximately 8 hours, transfer the cryovials to liquid nitrogen for long-term storage.

Note: Storage at -80°C should not exceed 48 hours.

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