cyagen | OriCell™

User Manual

OriCell™ SD Rat Bone Marrow Mesenchymal Stem Cells With GFP&Luciferase

Catalog No. RASMX-01401

We help you discover life

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

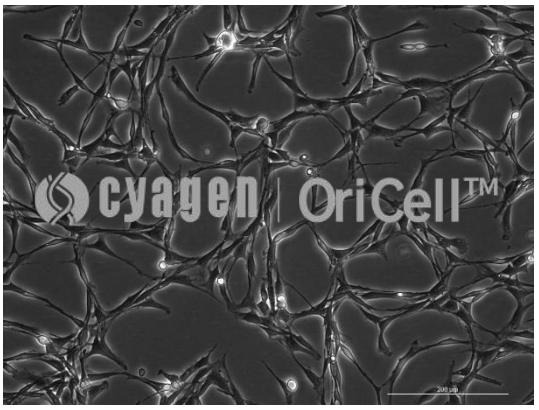
Note: **This product is only provided for further scientific research. It is not intended for diagnostic, therapeutic, clinical, household, or any other applications.**

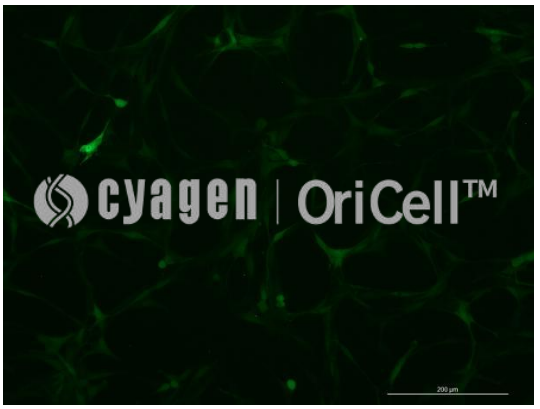
When citing our products in academic publications, please use the following format: "OriCell™ [Product Name] + [Catalog Number], from Cyagen Biosciences (Guangzhou) Inc."

Product Information

Name	OriCell™ SD Rat Bone Marrow Mesenchymal Stem Cells With GFP&Luciferase
Catalog Number	RASMX-01401
Amount of Cells	1×10^6 cells/vial
Passage Number	P5
Storage at	Liquid Nitrogen (-196°C)

The Morphology of OriCell™ SD Rat Bone Marrow Mesenchymal Stem Cells With GFP&Luciferase





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 Handing Principles

1. Ensure that all equipment is kept clean and tidy.
2. Please follow the instructions, strictly control the variables, and perform proper control experiments.
3. Use suitable and reliable consumables and reagents. This product requires culture vessels suitable for adherent cell growth, and their reuse is not recommended. The reagents used must be validated for reliability, suitable for cell growth, and exhibit minimal batch-to-batch variation.
4. Bone marrow mesenchymal stem cells have limited ability to proliferate in vitro and cannot maintain their differentiation potential for a long time. OriCell™ SD Rat Bone Marrow Mesenchymal Stem Cells With GFP&Luciferase 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 rat bone marrow mesenchymal stem cells is (2.5~4) $\times 10^4$ live cells/cm^2. The growth of these cells is strongly influenced by the donor's intrinsic characteristics as well as the subsequent culture system. We recommend adjusting the passage ratio according to the actual growth performance of each batch and passage.

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™ SD Rat Bone Marrow Mesenchymal Stem Cells With GFP&Luciferase (Cat. No.: RASMX-01401)
- 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.

   Note: Please calculate the corresponding rotational speed using the formula: $a=\omega^2 r$ (where a is the centripetal acceleration, ω is the angular velocity and r is the rotor radius. $\omega = \pi n/30$).

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 CO_2 incubator at 37°C with 5% CO_2 and saturated humidity.

    Note: **Do not disturb or observe the cells within the first 2 hours after seeding, as this may seriously 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, PBS 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 residual cells and add the wash to the centrifuge tube.
9. Centrifuge all collected cell suspensions at 250 × 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) × 10^4 live cells/cm^2, or adjust the seeding density based on the actual growth conditions of the cells.

    Note:

    The culture of OriCell™ SD Rat Bone Marrow Mesenchymal Stem Cells With GFP&Luciferase requires a relatively high cell density. We recommend manual cell counting when conditions permit and counting efficiency is high, in order to obtain an accurate cell concentration to guide seeding. If precise counting is not feasible, subculturing at an appropriate ratio is a better alternative. Typically, OriCell™ SD Rat Bone Marrow Mesenchymal Stem Cells With GFP&Luciferase are passaged at a ratio of 1:3, with cells reaching passage confluence within 72 hours. Please adjust the subculture ratio according to the actual condition of the cells.

12. Shake the cells gently and place them in an incubator at 37 °C, 5% CO_2, and saturated

humidity.

13. On the day after passaging, observe the cell condition. If a significant number of floating cells are present, replace the culture medium.
14. When cell confluence exceeds 95%, passage or cryopreserve the cells.

    Note: **Under normal conditions, the growth time of OriCell™ SD Rat Bone Marrow Mesenchymal Stem Cells With GFP&Luciferase 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.

    Note: **If accurate cell counting is not feasible, we recommend aliquoting cells proportionally for freezing. Prolonged storage under non-culture conditions will significantly reduce cell viability. Keep the cells at 4°C during counting to minimize metabolic activity and preserve cell integrity.**
5. When using any of the recommended NCR cryopreservation media above, cryovials can be directly placed 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.