



## User Manual

### OriCell™ C57BL/6 Mouse Bone Marrow

### Macrophage Cells

Catalog No. MUBMM-02051

## Introduction

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Macrophages originate from monocytes and are immune cells with diverse functions. They are non-proliferative and difficult to culture long-term. Macrophages play important roles in phagocytosis and clearance of foreign substances, as well as aged or dead cells, secretion of bioactive molecules, regulation of hematopoiesis, and participation in immune responses. As key immune effector cells, they contribute significantly to maintaining homeostasis and defending tissues.

OriCell™ C57BL/6 Mouse Bone Marrow Macrophage Cells are isolated from the femur and tibia of SPF-grade mice. These cells are generated by isolating bone marrow mononuclear cells and subsequently differentiating them into high-purity macrophages through in vitro culture with cytokine stimulation. They serve as a high-quality cellular model for studies in immunology, inflammation, and oncology.

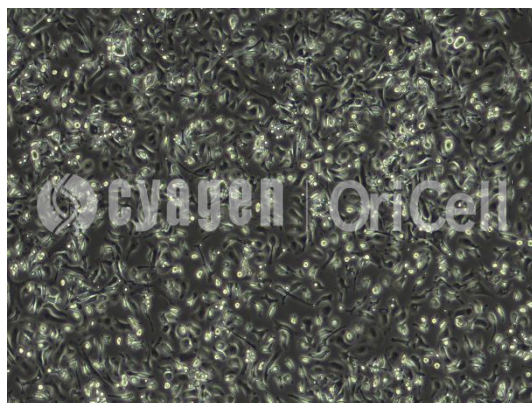
**Note:** This product is intended for research use only and is not for diagnostic, therapeutic, clinical, household, or any other applications.

When citing our products in academic publications, please use the following format: “OriCell™ [Product Name] + [Catalog Number], from Cyagen Biosciences.”

## Product Information

Product Name	OriCell™ C57BL/6 Mouse Bone Marrow Macrophage Cells
Catalog Number	MUBMM-02051
Cell Characteristics	Adherent growth; Macrophage-like
Passage Capability	Terminally differentiated; Non-proliferating
Identification Markers	F4/80 (by Flow Cytometry)
Number of Cells	1×10 <sup>6</sup> cells/vial
Passage Number	P0
Storage at	Liquid Nitrogen (-196°C)

### The Morphology of OriCell™ C57BL/6 Mouse Bone Marrow Macrophage Cells



## QC

- Pass the detection of bacteria, fungi, mycoplasma, and endotoxins.
- Verified by cell recovery viability testing, with a post-thaw survival rate >80%.
- Cell identification: F4/80 positive (>90%).

Please refer to "COA" for details.

## General Handling Principles

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1. Maintain strict aseptic technique. Ensure complete sterility throughout all procedures, particularly within the laminar flow hood and incubator.
2. Follow standardized protocols. Adhere strictly to the product manual. Implement rigorous control over experimental variables and include appropriate parallel controls.
3. Use high-quality consumables and reagents. This product requires culture vessels suitable for adherent cell growth, and the reuse of these vessels is not recommended. The reagents used must be validated for reliability, cell compatibility, and batch-to-batch consistency.
4. Optimize seeding density and subculture. The recommended seeding density for OriCell™ C57BL/6 Mouse Bone Marrow Macrophage Cells is  $(2.5-4) \times 10^4$  viable cells/cm<sup>2</sup>. Since cell growth is highly dependent on donor characteristics and culture conditions, we recommend adjusting the split ratios based on the actual performance of each specific lot and passage.

**Note:** The cryopreservation medium of this product contains DMSO, which may pose potential risks. Please handle it with care.

## Thawing and Culturing of Cells

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### Materials Required

- OriCell™ C57BL/6 Mouse Bone Marrow Macrophage Cells (Cat. No.: MUBMM-02051)
- OriCell™ Complete Medium For Mouse Bone Marrow Macrophage Cells (Cat. No.: MUBMM-90011)

### Steps

**Note:** If thawing is planned within 24 hours of receipt, store the cells in an ultra-low temperature freezer at -80 °C. For long-term storage (>24 hours), keep them in liquid nitrogen. Before thawing,

transfer the cells from liquid nitrogen to -80 °C and hold them there for 10 minutes. This will allow any residual liquid nitrogen to evaporate and prevent vial explosion.

1. Pre-warm the water bath to 37 °C.
2. Warm the complete medium to 37 °C.
3. Add at least 5 mL of pre-warmed 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 medium.

**Note:**

(1) Gently swirl 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 swirling the vial until it is completely thawed.

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  $250 \times g$  for 4 minutes.

**Note:** Please calculate the corresponding rotational speed using the formula:  $RCF = 1.118 \times 10^{-5} \times r \times RPM^2$  (where RCF is the relative centrifugal force,  $r$  is the rotor radius in cm, and RPM is the rotational speed).

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. Seed 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<sub>2</sub> incubator at 37 °C with 5% CO<sub>2</sub> and saturated humidity.

**Note:** Do not move or observe the cells within the first 2 hours after seeding, as this may impair cell adhesion, causing poor morphology, clumping, and uneven attachment.

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

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

13. Replace the complete medium every 2 days until the cells reach a stable growth phase, at which point they are ready for downstream experiments.

**Note:** Do not store the cells at -80 °C for more than 48 hours.

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