



# User Manual

## OriCell™ C57BL/6 Mouse Cortical

### Astrocytes

Catalog No. BCCAC-00001

## Introduction

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Astrocytes are star-shaped glial cells found in the brain and spinal cord. They play a crucial role in the formation of the blood-brain barrier (BBB), supplying nutrients to neural tissues, maintaining extracellular ionic balance, and facilitating the repair of brain and spinal cord injuries.

As the most widely distributed and largest type of glial cell in the mammalian brain, astrocytes possess distinct morphological characteristics. When visualized using classic metal impregnation techniques (silver staining), these cells exhibit a stellate morphology with numerous long, branched processes extending from the cell body. These processes fill the spaces between neuronal cell bodies and their projections, thereby providing structural support and isolating neurons. The ends of these processes often expand to form endfeet (also known as pedicels). Some of these endfeet, attaching to the walls of adjacent capillaries, are known as "vascular feet" or "perivascular endfeet". Meanwhile, those near the surface of the brain and spinal cord attach to the inner surface of the pia mater, connecting to form the glia limitans.

OriCell™ C57BL/6 Mouse Cortical Astrocytes are isolated from the cerebral cortex of neonatal mice, retaining these characteristic morphological and functional properties. These cells provide a reliable in vitro model for investigating astrocyte biology and neurovascular regulation, as well as for studying BBB formation, neurotrophic support, and mechanisms of CNS (central nervous system) injury repair.

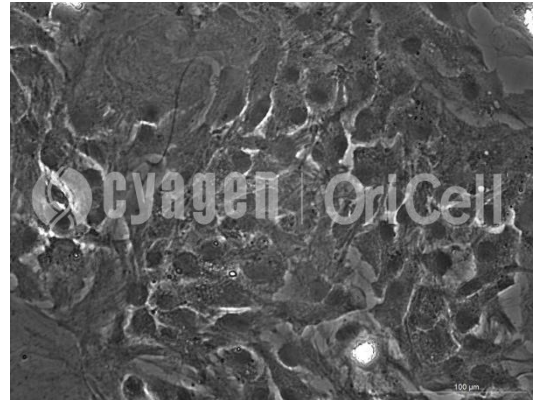
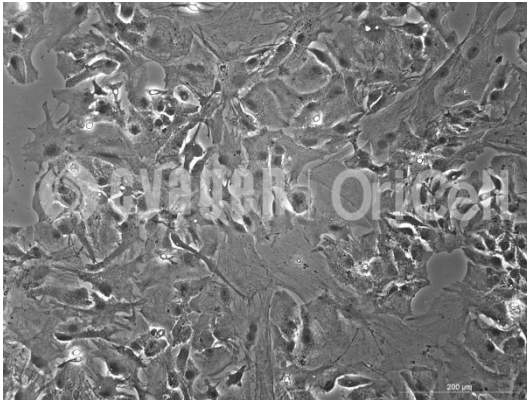
**Note:** This product is intended for research use only and is not for diagnostic, therapeutic, clinical, household, or any other applications.

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 Cortical Astrocytes
Catalog Number	BCCAC-00001
Amount of Cells	1×10 <sup>6</sup> cells/vial
Passage Number	P2
Storage at	Liquid Nitrogen (-196°C)

### The Morphology of OriCell™ C57BL/6 Mouse Cortical Astrocytes



## QC

- Pass the detection of bacteria, fungi, mycoplasma, and endotoxins.
- Verified by cell recovery viability testing, with a post-thaw survival rate >80%.
- Verified by cell cycle analysis, with a doubling time < 72 h.
- Verified by immunofluorescence (IF): Positive for GFAP (≥ 80%); Negative for β-tubulin III (≤ 10%) and Galc (≤ 10%).

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. Prioritize low-passage cells. Generally, cortical astrocytes have limited ability to proliferate in vitro and cannot maintain their differentiation potential for a long time. Leveraging our extensive cell culture expertise and optimized culture systems, OriCell™ C57BL/6 Mouse Cortical Astrocytes can be subcultured for more than 3 passages while maintaining their phenotypic integrity and meeting our rigorous quality control standards. However, we always recommend using low-passage cells for research applications.
5. Optimize seeding density and subculture. The recommended seeding density for OriCell™ C57BL/6 Mouse Cortical Astrocytes is  $(3-4) \times 10^4$  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.
6. Refreezing of the cells after thawing is not recommended.

**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 Cortical Astrocytes (Cat. No.: BCCAC-00001)
- OriCell™ Complete Medium For Mouse Cortical Astrocytes (Cat. No.: MUXAC-90011)

### Steps

**Note:** If thawing is planned within 24 hours of receipt, store the cells in an ultra-low temperature freezer at  $-80^{\circ}\text{C}$ . For long-term storage (>24 hours), keep them in liquid nitrogen. Before thawing, transfer the cells from liquid nitrogen to  $-80^{\circ}\text{C}$  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  $37^{\circ}\text{C}$ .
2. Warm the complete medium to  $37^{\circ}\text{C}$ .
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  $-80^{\circ}\text{C}$  freezer, immerse it in the  $37^{\circ}\text{C}$  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 approximately 90% confluence, at which point they are ready for passage.

## Passaging of Cells

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### 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 Mouse Cortical Astrocytes (Cat. No.: MUXAC-90011)

### Steps

1. Pre-warm the complete medium, PBS and trypsin to 37°C.
2. Remove the medium in the culture vessel.
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 creating excessive bubbles, as this may cause cell damage or loss.

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  $250 \times g$  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. Seed the cells into a suitable culture vessel at a density of  $(3-4) \times 10^4$  viable cells/cm<sup>2</sup>, or adjust the seeding density based on the actual growth conditions of the cells.

**Note:** We recommend manual cell counting where possible to obtain an accurate concentration for seeding. If precise counting is not feasible, subculturing at an appropriate ratio is a reliable alternative. Typically, OriCell™ C57BL/6 Mouse Cortical Astrocytes are passaged at a ratio of 1:3, with cells reaching confluence within 72 hours. Please adjust the subculture ratio based on the actual growth of the cells.

12. Gently agitate the vessel to ensure uniform cell distribution and place it in an incubator at 37 °C, 5% CO<sub>2</sub>, 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. Replace the culture medium every 2 days. When cells reach 90% confluence, passage or cryopreserve the cells.

**Note:** Under normal conditions, the growth time of OriCell™ C57BL/6 Mouse Cortical Astrocytes does not exceed 72 hours per generation, and there is no need to change the medium. Frequent medium replacement may disrupt the established cellular microenvironment.

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