cyagen | OriCell™

# User Manual

## OriCell™ SD Rat Cortical Astrocytes

Catalog No. SCCAC-00001

*We help you discover life*

## Introduction

Astrocytes are a type of stellate glial cell found in the brain and spinal cord. They play a crucial role in the formation of the blood-brain barrier. Astrocytes nourish neural tissue, maintain extracellular ion balance, and participate in the repair of brain and spinal cord injuries.

Cortical astrocytes are valuable for neurobiological research, including maintaining the extracellular environment of the central nervous system, supporting neuronal metabolism and neurotransmitter synthesis, and facilitating the research and development of new drugs for Parkinson's disease and Alzheimer's disease.

OriCell™ SD Rat Cortical Astrocytes are isolated from the cerebral cortex of newborn SD rats.

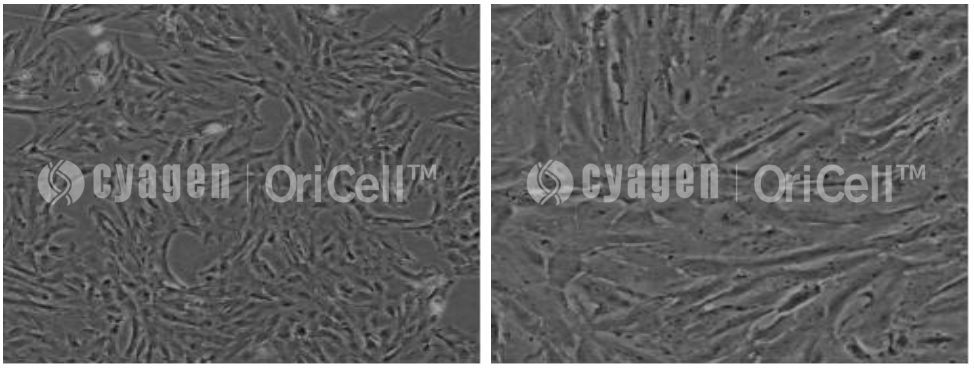
Note: **This product is only provided for further scientific research. It is not intended for diagnostic, therapeutic, clinical, household, or any other applications.**

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

## Product Information

| | |
|---|---|
| Name | OriCell™ SD Rat Cortical Astrocytes |
| Catalog Number | SCCAC-00001 |
| Amount of Cells | $1\times10^6$ cells/vial |
| Passage Number | P2 |
| Storage at | Liquid Nitrogen (-196°C) |

The Shape of OriCell™ SD Rat Cortical Astrocytes



## QC

- Pass the detection of bacteria, fungi, mycoplasma and endotoxin.
- Pass the cell resuscitation test, the resuscitation survival rate is >50%.
- Pass the cell cycle detection, the doubling period is less than 72 h.
- Pass the immunofluorescence detection, it expresses GFAP (≥ 80%). But it does not express β-tubulin III ≤ 10%, Galc (≤ 10%) .

**Please refer to "COA" for details.**

## General Handing Principles

1. Ensure that all equipment is kept clean and tidy.
2. Please follow the instructions. Please operate according to the method described in the product manual, strictly control the variables, and do a controlled experiment.
3. Use suitable and reliable consumables and reagents.This product needs to use a culture container suitable for the growth of adherent cells, and it is not recommended to reuse it. The reagents used must be validated, reliable, suitable for cell growth and have small batch-to-batch variation.

4. Cortical astrocytes have limited ability to proliferate in vitro and cannot maintain their differentiation potential for a long time. Based on the rich cell culture experience and excellent performance of the culture system, OriCell™ SD Rat Cortical Astrocytes can be passaged in vitro for more than 3 times and still maintain all indicators qualified. However, we always recommend using lower generation cells for scientific research as much as possible.
5. OriCell™ SD Rat Cortical Astrocytes are usually inoculated at a density of (3~4) × $10^4$ viable cells/$cm^2$. The growth of this cell has a great relationship with the donor's own characteristics and the subsequent culture system.
6. It is not recommended to cryopreserve the cells and reuse it .

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 Cortical Astrocytes (Cat. No.: SCCAC-00001)
- OriCell™ Complete Medium For Rat Cortical Astrocytes (Cat. No.: RAXAC-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 Rat Cortical Astrocytes (Cat. No.: RAXAC-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 (3~4)×$10^4$ live cells/$cm^2$, or adjust the passage ratio according to the actual growth of the cells.

    Note: **OriCell™ SD Rat Cortical Astrocytes 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 SD Rat Cortical Astrocytes 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.**

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