cyagen | OriCell™

# User Manual

## OriCell™ SD Fetal Rat Cortical Neurons

**Catalog No. SCCFN-00001**

*We help you discover life*

## Introduction

Neurons, a type of nerve cell, connect with each other to form neural networks. They are the main components of the nervous system and serve as effective models for studying its function. Neurons are widely used in neurobiological research and the development of new treatments for Parkinson's disease and Alzheimer's disease.

OriCell™ SD Fetal Rat Cortical Neurons are derived from the cerebral cortex tissue of Sprague-Dawley (SD) fetal rats at embryonic day 18.5 (E18.5), and are cryopreserved at the primary culture stage. They express specific neuronal proteins and can be used as a cell model for research in proliferation, aging, immunity, and other related fields.

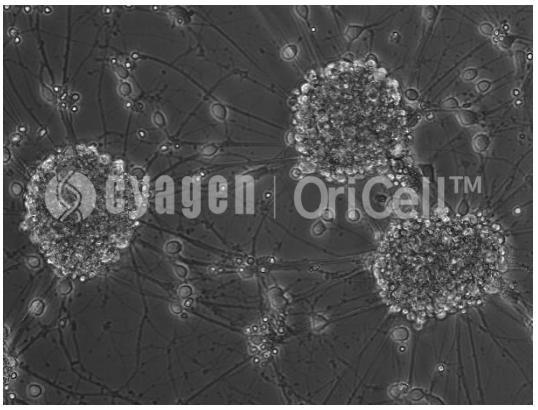
Note: **This product is only provided for further scientific research. It is not intended for diagnostic, therapeutic, clinical, household, or any other applications.**

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

## Product Information

| | |
|---|---|
| Name | OriCell™ SD Fetal Rat Cortical Neurons |
| Catalog Number | SCCFN-00001 |
| Amount of Cells | $2\times10^6$ cells/vial |
| Passage Number | P0 |
| Storage at | Liquid Nitrogen (-196°C) |

The Shape of OriCell™ SD Fetal Rat Cortical Neurons


## QC

- Pass the detection of bacteria, fungi, mycoplasma and endotoxin.
- Pass the cell resuscitation test, the resuscitation survival rate is >50%.
- Pass the immunofluorescence detection, it expresses MAP2 and β-tubulin III (>70%). But it does not express GFAP (<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. OriCell™ SD Fetal Rat Cortical Neurons are usually inoculated at a density of (1~2) × $10^5$ viable cells/$cm^2$. The growth of this cell has a great relationship with the donor's own characteristics and the subsequent culture system.

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

## Preparation of Culture Vessel (Coated with PLL and Laminin)

### Materials Required

- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No.: PBS-10001)
- Cell culture plate (take 24-well plate as an example)
- Poly-L-lysine, PLL
- Laminin

### Steps

1. Prepare PLL/Laminin-coated plates at least one day before the cell differentiation experiment.
2. Use ultrapure water to dilute PLL to a working solution with a final concentration of 15 μg/mL for use.
3. Add 0.5 mL of the above PLL working solution to each well of the 24-well plate to evenly cover the bottom surface.
4. Put it at room temperature for at least 30 minutes.
5. Aspirate the PLL, and gently rinse once with 1 mL of ultrapure water.
6. Dilute Laminin with PBS to a final concentration of 15 μg/mL working solution for later use.
7. Add 0.5 mL of the above Laminin working solution to the culture plate, evenly cover the bottom surface, seal with a parafilm, and place it at 4°C overnight for later use.

   Note: **1）When PLL and Laminin are coated, there should be no air bubbles remaining on the**

bottom of the well plate;

2) Laminin coated culture plates can be placed at 4°C for a week, please use within a week.

8. Aspirate Laminin before inoculating cells, wash it with PBS once, and dry it for later use.

## Thawing and Establishing of Cells

### Materials Required

- OriCell™ SD Fetal Rat Cortical Neurons (Cat. No.: SCCFN-00001)
- OriCell™ Serum Free Medium For Rat Neurons (Cat. No.: RAXFN-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 2 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. When shaking, please avoid water immersing the pipe cover to cause pollution.

5. 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.
6. Wipe the outer surface of the cryotube with 75% ethanol.

7. Open the cryopreservation tube in the ultraclean bench, use a pipette to take a small amount (about 0.5 mL) of complete neuron culture medium, add dropwise to the cryopreservation tube, and gently pipette several times.
8. Use a pipette to add the cryopreserved suspension of all cells drop by drop to a centrifuge tube with 2 mL of neuron complete medium, and gently pipette several times.

   Note: **The process of pipetting must be gentle, and no air bubbles are allowed to avoid damage to the cells.**

9. After counting the cells, inoculate the cells into the PLL-Laminin-coated culture plate at $2 \times 10^5$ viable cells/$cm^2$ (1 tube of OriCell™ SD Fetal Rat Cortical Neurons can inoculate about 4% wells of the 24-well plate). Add a sufficient amount of complete medium, and the total amount of medium in each well of the plate should more than 0.5 mL.
10. Shake the cells well and incubate them in a $CO_2$ incubator at saturated humidity, 37°C, 5% $CO_2$ inside.
11. 6h after resuscitation, replace with fresh complete medium, making sure to make gentle movements.
12. Semi-refresh the medium every 2 days ( aspirate 250 uL of medium and add 250 uL of medium). If there are too many dead cells, it is recommended to change the medium every day.

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