cyagen | OriCell

# User Manual

## OriCell™ Wistar Fetal Rat Hippocampal Neurons

**Catalog No. WHCFN-00001**

*We help you discover life*

## Introduction

Neurons, a type of nerve cell, connect with each other to form neural networks. As the primary functional units of the nervous system, they serve as essential 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™ Wistar Fetal Rat Hippocampal Neurons are derived from the cerebral cortex tissue of 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 on proliferation, aging, immunity, and other related fields.

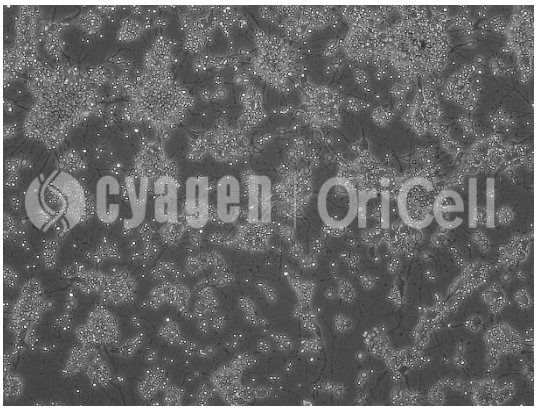
Note: **This product is intended for research use only and is not for diagnostic, therapeutic, clinical, household, or any other applications.**

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™ Wistar Fetal Rat Hippocampal Neurons |
| Catalog Number | WHCFN-00001 |
| Amount of Cells | 1 ×$10^6$ cells/vial |
| Passage Number | P0 |
| Storage at | Liquid Nitrogen (-196°C) |

The Morphology of OriCell™ Wistar Fetal Rat Hippocampal Neurons


## QC

- Pass the detection of bacteria, fungi, mycoplasma, and endotoxins.
- Verified by cell recovery viability testing, with a post-thaw survival rate >50%.
- Verified by immunofluorescence (IF): Positive for MAP2 and β-tubulin III (> 70%); Negative for GFAP (< 10%).

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

## General Handing Principles

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™ Wistar Fetal Rat Hippocampal Neurons is (1–2) × $10^5$ viable cells/$cm^2$. 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.**

## 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. Dilute PLL with ultrapure water to a final concentration of 15 μg/mL to prepare the working solution.
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. Leave the plate 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 to prepare the working solution.
7. Add 0.5 mL of the above Laminin working solution to each well, ensuring even coverage. Seal the

plate with Parafilm and store at 4 °C overnight.

Note:

1) Avoid Bubbles: Ensure no air bubbles remain on the bottom of the wells during the PLL and Laminin coating process.
2) Storage: Laminin-coated plates can be stored at 4 °C for up to one week. Please use them within this timeframe.

8. Aspirate the Laminin, wash once with PBS, and allow the wells to air-dry before seeding the cells.

## Thawing and Culturing of Cells

### Materials Required

- OriCell™ Wistar Fetal Rat Hippocampal Neurons (Cat. No.: WHCFN-00001)
- OriCell™ Serum Free Medium For Rat Neurons (Cat. No.: RAXFN-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 2 mL of pre-warmed complete medium to a 15 mL centrifuge tube for subsequent use.
4. Remove the cryovial from the −80 °C freezer, immerse it in the 37 °C water bath, and gently

swirl to thaw the cryopreservation medium quickly.

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. Use a Pasteur pipette to take a small amount (approximately 0.5 mL) of complete medium (Cat. No.: RAXFN-90011) and add it dropwise into the cryovial. Gently pipette up and down to mix thoroughly.
7. Add the entire cell suspension dropwise into the prepared centrifuge tube containing 2 mL of complete medium. Gently pipette up and down to mix thoroughly.

   Note: **Pipetting should be performed gently to avoid creating excessive bubbles, as this may cause cell damage or loss.**

8. After counting the cells, seed them onto the PLL/Laminin-coated plates at a density of $2 \times 10^5$ viable cells/$cm^2$. Add a sufficient volume of complete medium to ensure the final volume in each well is at least 0.5 mL.
9. Gently pipette to evenly distribute the cells, then place the plate in a $CO_2$ incubator at 37 °C with 5% $CO_2$ and saturated humidity.
10. At 6 hours post-seeding, replace the medium with fresh complete medium. Perform this step gently.
11. Thereafter, perform a half-medium change every 2 days (i.e., aspirate 250 µL of old medium and replace with 250 µL of fresh medium). If significant cell death is observed, change the medium daily.