cyagen | OriCell

User Manual

OriCell™ SD Rat Neural Stem Cells

Catalog No. RASNF-01001

We help you discover life

Introduction

Neural stem cells are pluripotent stem cells that can differentiate into various cell types of the nervous system, including neurons, astrocytes, oligodendrocytes, etc. Neural stem cells can be isolated from different regions of the mammalian brain, spinal cord, and other parts of the nervous system. Their capacity to rebuild neural circuits endows them with the potential for repairing brain tissue damage. Therefore, they hold extensive research value in animal models of neurodegenerative diseases, hereditary central nervous system disorders, stroke, and spinal cord injury.

OriCell™ SD Rat Neural Stem Cells are derived from the hippocampus of 14.5-day SD rats, cultured as suspended neurospheres, and cryopreserved at low passages. They can be used as a cell model to study proliferation, aging, immune responses, differentiation, and transplantation.

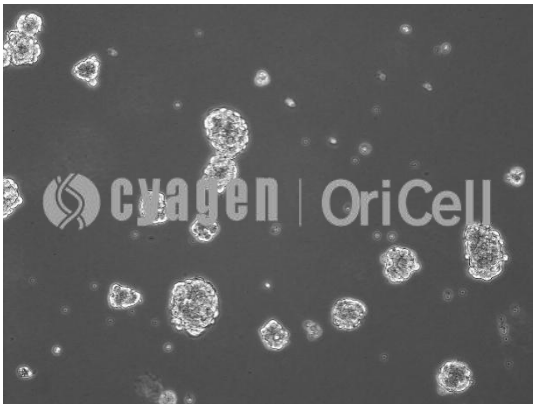
Note: **This product is intended for research use only and is not for diagnostic, therapeutic, clinical, household, or any other applications.**

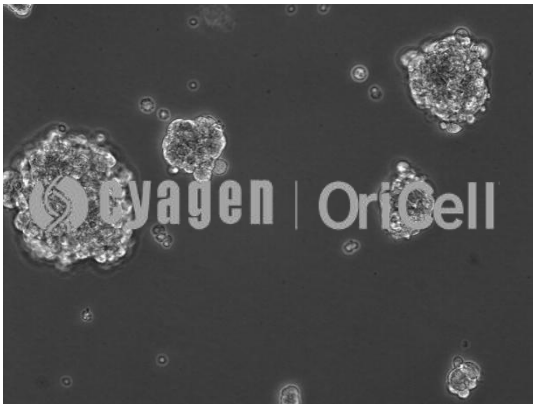
When citing our products in academic publications, please use the following format: "OriCell™ [Product Name] + [Catalog Number], from Cyagen Biosciences."

Product Information

Product Name	OriCell™ SD Rat Neural Stem Cells
Catalog Number	RASNF-01001
Amount of Cells	1×10^6 cells/vial
Passage Number	P2
Storage at	Liquid Nitrogen (-196 °C)

The Morphology of OriCell™ SD Rat Neural Stem Cells





QC

- Pass the detection of bacteria, fungi, mycoplasma, and endotoxins.
- Verified by cell recovery testing, with a post-thaw viability of > 50%.
- Verified by cell cycle analysis, with a doubling time < 72 h.
- Verified by immunofluorescence: Positive for Nestin (> 75%); Negative for GFAP and Tubulin (< 10%).
- Proven differentiation potential into neurons, oligodendrocytes, astrocytes, etc.

Please refer to "COA" for details.

General Handling 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

suspension 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, neural stem cells 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™ SD Rat Neural Stem Cells can be subcultured for more than 3 passages while 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™ SD Rat Neural Stem Cells is (1.5–3) × 10^5 viable cells/mL. 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 has potential risks. Please handle it carefully.**

Thawing and Culturing of Cells

Materials Required

- OriCell™ SD Rat Neural Stem Cells (Cat. No.: RASNF-01001)
- OriCell™ Serum Free Medium For Rat Neural Stem Cells (Cat. No.: RAXNF-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 160 × g for 5 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 culture vessel at a density of (1.5~3) × 10^5 viable cells/mL, add a sufficient amount of complete medium, and mix by pipetting.
11. Incubate the cells in a CO_2 incubator at 37 °C with 5% CO_2 and saturated humidity.

    Note: **Do not move or observe the cells within 12 hours of inoculation, as this may cause**

uneven cell distribution and neurosphere aggregation/fusion with variable size and morphology.

12. On the day after recovery (thawing), observe the cell status and replace with fresh complete medium or proceed with passaging.

    Note: Neural stem cells are sensitive to temperature. Low room temperature and prolonged observation time may lead to aggregation and fusion of neurospheres. If you find abnormal conditions, please investigate the cause in time and contact us.

Changing Cell Culture Medium

Materials Required

- OriCell™ Serum Free Medium For Rat Neural Stem Cells (Cat. No.: RAXNF-90011)

Steps

Note: To avoid repeated heating, aliquot the medium into sterile containers if it cannot be used at once. Only prewarm the required volume before use.

1. Observe the cells under a microscope. If adherent cells are present, avoid tapping the culture vessel to prevent detachment.
2. Transfer the neurosphere suspension to a centrifuge tube using a Pasteur pipette.
3. Centrifuge the suspension at 140 × g for 4 minutes, then aspirate and discard the supernatant.
4. Add 1 mL of complete medium (Cat. No.: RAXNF-90011) to the cell pellet, and gently resuspend the cells without disrupting the small neurospheres.
5. Transfer the cell suspension into a new culture vessel, add the appropriate volume of complete medium, and mix gently by pipetting.

6. Incubate the cells in a CO_2 incubator at 37 °C with 5% CO_2 under saturated humidity.
7. Subsequently, perform medium changes or cell passage based on cell growth and culture conditions. Generally, medium change is unnecessary in the absence of significant cell debris, dead cells, or cell adherence.

   Note: **Neural stem cells must be passaged immediately if large neurospheres develop dark or dim centers, or if signs of adherent differentiation are observed. Under standard culture conditions, SD rat neural stem cells are typically passaged every 2–3 days.**

Passaging of Cells

Materials Required

- OriCell™ Serum Free Medium For Rat Neural Stem Cells (Cat. No.: RAXNF-90011)
- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No.: PBS-10001)

Steps

1. Prewarm the complete medium to 37 °C.
2. Observe the cells under a microscope. If adherent cells are present, avoid tapping the culture vessel to prevent detachment.
3. Transfer the neurosphere suspension to a centrifuge tube using a Pasteur pipette.
4. Gently rinse the culture vessel once with PBS and add the wash to the centrifuge tube.
5. Centrifuge the suspension at a reduced speed of 160× g for 4 minutes, then aspirate and discard the supernatant.
6. Resuspend the cell pellet in 1 mL of complete medium (Cat. No.: RAXNF-90011) and transfer the suspension to a 5 mL microcentrifuge tube (e.g., Eppendorf tube). Gently triturate the pellet 15–20 times using a 1 mL pipette tip. Optimal dissociation is achieved when the suspension primarily consists of single cells or small clusters of 2–3 cells. (If the cell pellet is large, divide the suspension

evenly into 2–3 tubes prior to trituration).

Note: **Perform trituration gently to avoid generating bubbles, which can cause cell damage and reduce viability.**

7. Allow the EP tube to stand undisturbed for 1–2 minutes to let undissociated aggregates settle.
8. Collect the cells and seed them into T25 culture flasks. Add an appropriate volume of complete medium, and adjust the seeding density to (1.5~3) × 10^5 live cells/mL. Mix gently by pipetting up and down.

   Note: **Do not disturb or aspirate the cell pellet/aggregates remaining at the bottom of the tube, as these are not recommended for further culture.**

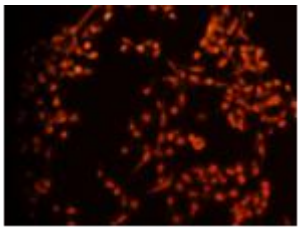
9. Invert or swirl the flask gently to distribute the cells evenly, then incubate in a CO_2 incubator at 37 °C with 5% CO_2 under saturated humidity.
10. On the day following passage (Day 1), perform a medium change if a significant amount of cell debris, dead cells, or unexpected cell adherence is observed.

Identification of Cells

Neural stem cells are cultured in serum-free medium supplemented with EGF and FGF. Upon removal of mitogens, the cells spontaneously differentiate into neurons, astrocytes, and oligodendrocytes. In addition to spontaneous differentiation, neural stem cells can also be directed toward specific lineages under defined induction conditions. When cultured in serum-containing medium for approximately 7 days, neural stem cells spontaneously differentiate into neurons (16 ± 7%), astrocytes (75 ± 7%), and oligodendrocytes (5 ± 3%).

Immunofluorescence Staining of Neural Stem Cells for Purity Analysis

Neural stem cell marker Nestin (Red), astrocyte marker GFAP (Negative), neuron-specific marker Tubulin (Negative), and Hoechst (Blue).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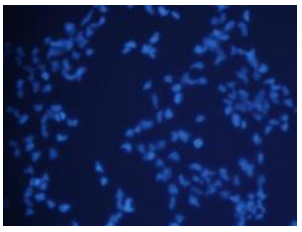
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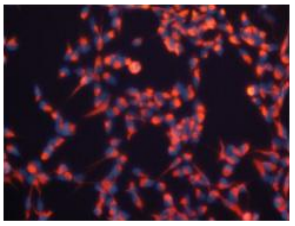
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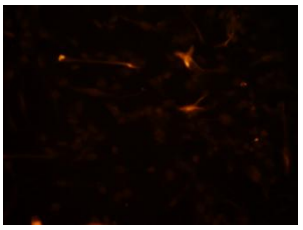
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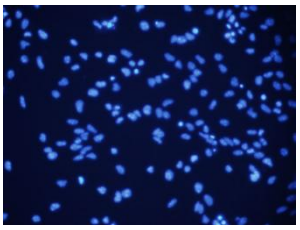
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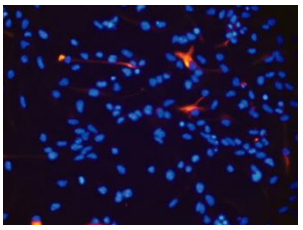
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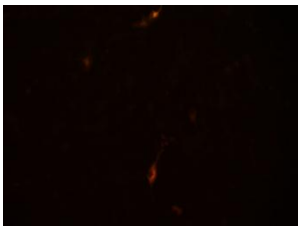
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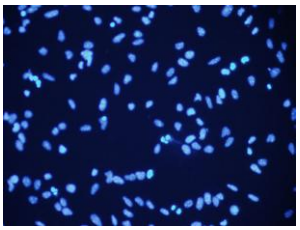
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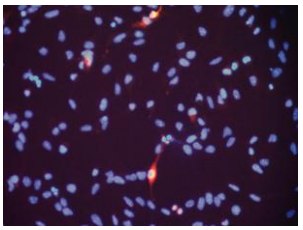
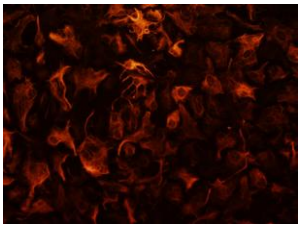

P4-merge-400×

Immunofluorescence Analysis of Induced Differentiation of Neural Stem Cells

Astrocyte marker GFAP (Red), neuronal marker Tubulin (Red), and Hoechst (Blue).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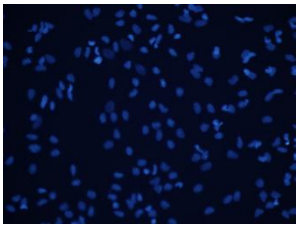
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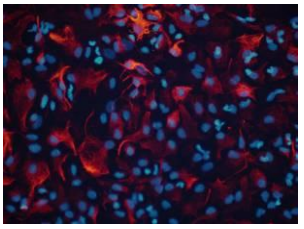
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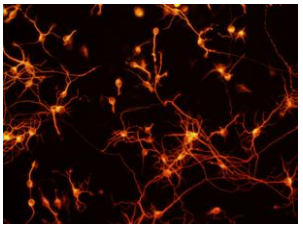
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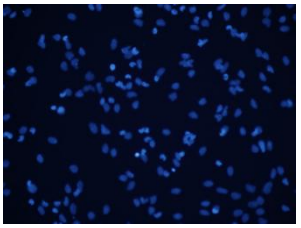
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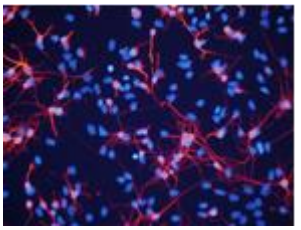
P4-merge-400×

Cryopreservation of Cells

Materials Required

- OriCell™ Protein-free Cryopreservation Medium For Neural Stem Cells (Cat. No.: GUXNX-07031)
- OriCell™ NCR Protein-free Cryopreservation Medium For Neural Stem Cells (Cat. No.: GUXNX-07021)

Note: **OriCell™ Protein-free Cryopreservation Medium for Neural Stem Cells is a protein-free, ready-to-use cryopreservation medium specifically designed for neural stem cells. It features a defined chemical composition and enables direct storage at -80 °C without controlled-rate freezing.**

Steps

1. For cell collection, please refer to Steps 1–8 of the OriCell™ SD Rat Neural Stem Cell Passage Protocol. (Note: Collect the upper cell suspension in Step 8, but do not seed it into T25 flasks, then proceed directly to the following steps).
2. Following collection, take a small aliquot of the cell suspension and count using a hemocytometer to determine the total cell number.
3. Centrifuge the suspension at 160 × g for 4 minutes, then aspirate and discard the supernatant.
4. Resuspend the cell pellet in the cryopreservation medium and adjust the density to 1×10^6 viable cells/mL (or your target density).
5. Aliquot the cell suspension into cryovials based on your desired cell number or ratio per vial.

Note: If standard cell counting equipment is unavailable, we recommend dividing the cells proportionally. Long-term suspension under non-culture environments will severely compromise cell viability. While counting, store the remaining cell suspension at 4 °C to reduce cellular metabolism and maintain cell health.

6. If you choose OriCell™ Protein-free Cryopreservation Medium For Neural Stem Cells (Cat. No.: GUXNX-07031), place the cryovials into a pre-cooled controlled-rate freezing container and transfer it to a -80 °C freezer. If you choose OriCell™ NCR Protein-free Cryopreservation Medium For Neural Stem Cells (Cat. No.: GUXNX-07021), place the cryovials directly into the -80 °C freezer.

   Note: To ensure optimal cell survival, do not open the freezer door during the initial freezing process, especially within the first 4 hours.

7. After 24 hours, transfer the cryovials to liquid nitrogen for long-term storage.

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