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 a variety of cells of the nervous system, including neurons, astrocytes, oligodendrocytes, et al. Neural stem cells can be isolated from different regions of the mammalian brain and the spinal cord and other nervous systems. Neural stem cells have the ability to rebuild neural circuits, so they have the potential to treat brain tissue damage, and thus have extensive research value in animal models of neurodegenerative diseases, hereditary central nervous system diseases, stroke and spinal cord injury.

OriCell™ SD Rat Neural Stem Cells are derived from the brain tissue of 14.5 days old SD rats and are cultured in the form of suspended neurospheres. It was cryopreserved at low passages. It can be used as a cell model to study proliferation, aging, immunity, differentiation and transplantation.

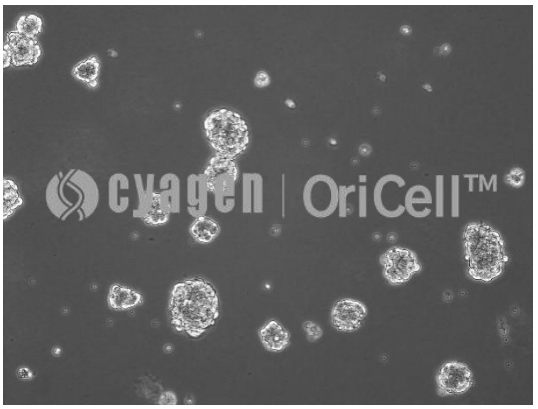
Note: **This product is only provided for further scientific research. It is not intended for diagnostic, therapeutic, clinical, household, or any other applications.**

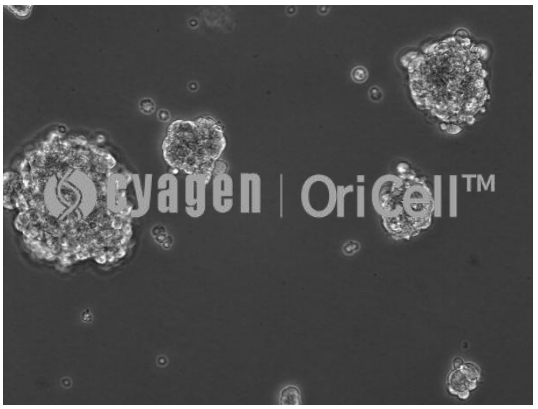
When citing our products in academic journals, please indicate "OriCell™ + Catalog Number, from Cyagen Biosciences (Guangzhou) Inc."

Product Information

Name	OriCell™ SD Rat 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 Shape of OriCell™ SD Rat Neural Stem Cells





QC

- Pass the detection of bacteria, fungi, mycoplasma, and endotoxins.
- 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 Nestin (> 75%), but does not express GFAP and Tubulin (< 10%).
- Have good differentiation potential, can differentiate into neurons, oligodendrocytes, astrocytes, etc.

Please reference "COA" for details.

General Handing Principles

1. Ensure that all equipment is kept clean and tidy.
2. Please follow the instructions.
3. Use suitable and reliable consumables and reagents.
4. Neural stem cells have limited ability to proliferate in vitro and cannot maintain their differentiation potential for a long time. Based on rich cell culture experience and excellent performance culture system, OriCell™ SD Rat Neural Stem Cells can be passaged more than 3 times in vitro and still

maintain all indicators qualified. However, we always recommend using lower generation cells for scientific research as much as possible.

5. The usual inoculation density of neural stem cells in SD rat is (1.5~3) $\times 10^5$ viable cells/mL. The growth of this cell has a great relationship with the donor's own characteristics and the subsequent culture system. We recommend subculture according to the actual growth conditions of each batch and each generation.

   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 Neural Stem Cells (Cat. No. RASNF-01001)
- OriCell™ Serum Free Medium For Rat Neural Stem Cells (Cat. No. RAXNF-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 160×g for 5 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 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.
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 12 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: **Neural stem cells are sensitive to temperature. Low room temperature and long observation time will cause neurospheres to adhere to each other. If you find lots of floating cells or other 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 repeatedly warming the culture medium, it is recommended to dispense the medium into suitable aseptic containers if it cannot be used all at once. When changing the medium, only preheat the amount needed for the day.**

1. Observe the cells under the microscope. If there are cells that adhere to the wall, try not to tap the culture vessel to prevent them from falling off.
2. Use a Pasteur pipette to transfer the neurosphere suspension to a centrifuge tube.
3. Reduce the centrifugal force to 140×g, centrifuge the neurosphere suspension for 4 min, and then remove the supernatant.
4. Add 1 mL of neural stem cell complete medium to the cell pellet, and gently resuspend the cells, do not to blow the small neurospheres apart.
5. Transfer the cell suspension into a new culture vessel, add enough complete medium, and mix by pipetting.
6. Incubate cells in a CO_2 incubator at saturated humidity, 37°C, 5% CO_2 inside.
7. After that, depending on the condition of the culture medium and the growth of the cells, carry out the operation of changing the medium or the passage of the cells. Generally, when there is no large number of dead cells, cell debris and cell adhesion, there is no need to change the medium.

   Note: **Neural stem cells need to be passaged immediately when they have large neurospheres (the center of the neurospheres are dim/dark) or some of the neurospheres appear adherent differentiation. Under normal circumstances, SD rat neural stem cells are passaged once every 2 to 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 the microscope. If there are cells that adhere to the wall, try not to tap the culture vessel to prevent them from falling off.
3. Use a Pasteur pipette to transfer the neurosphere suspension to a centrifuge tube.
4. Gently wash the culture container once with PBS. After washing is completed, collect the PBS.
5. Reduce the centrifugal force to 160×g, centrifuge the neurosphere suspension for 4 minutes, and then remove the supernatant.
6. Add 1 mL of neural stem cell complete medium to the cell pellet to resuspend the cells, transfer the cell suspension to a 5 mL EP tube, and gently blow the cell pellet with a 1 mL pipette tip about 15-20 times. When the cells appear as a single or two or three clusters, it indicates that the dispersion effect is good. (When the amount of cells is large, the cell suspension can be divided evenly into 2~3 EP tubes and then pipetted).

   Note: **The pipetting action should not be violent, to avoid generating a lot of bubbles, otherwise it may damage and lose cells.**

7. Let the EP tube stand for 1 to 2 minutes.
8. Collect the cells, inoculate them into T25 culture flasks, gently pipette to mix, add sufficient neural stem cell complete medium, and adjust the inoculation density to (1.5~3) × 10^5 live cells/mL.

   Note: **Do not aspirate the cell cluster at the bottom of the EP tube. This part of the cell is not recommended to use.**

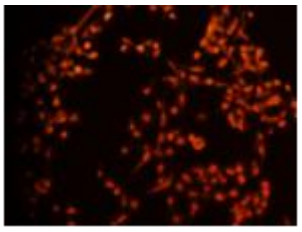
9. Shake the cells well and incubate them in a CO_2 incubator at saturated humidity, 37°C, 5% CO_2 inside.
10. On the next day of passaging, if a large number of dead cells, cell debris and cell adherence appear, the medium needs to be replaced.

Identification of cells

Neural stem cells are cultured in serum-free medium containing EGF and FGF. After the mitogen is removed, neural stem cells will spontaneously differentiate into neurons, astrocytes, and oligodendrocytes. In addition to spontaneous differentiation, neural stem cells can differentiate in the induced direction under specific culture conditions. Neural stem cells cultured in serum-containing medium for about 7 days, they will spontaneously differentiate into neuronal cells (16±7%), astrocytes (75±7%), and oligodendrocytes (5±3%) .

The Immunofluorescence Picture of Neural Stem Cells under Purity Detection

Neural Stem Cell Specific Protein Nestin (Red), Astrocyte Specific Protein GFAP (Negative), Neuron-specific Protein Tubulin (Negative), Hochest (Blue)



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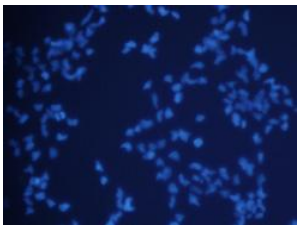

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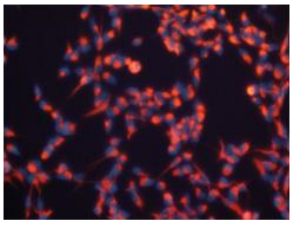
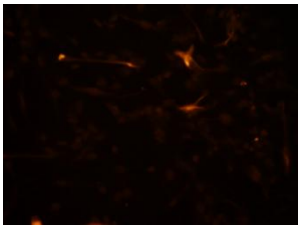
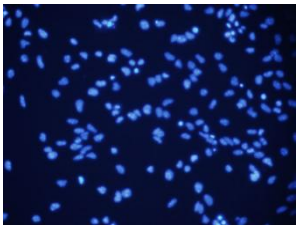


P4-merge-400×



P4-GFAP-400×



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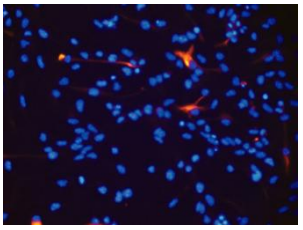

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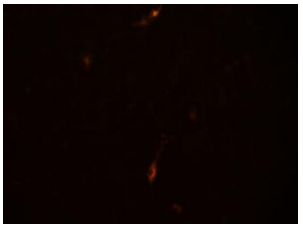
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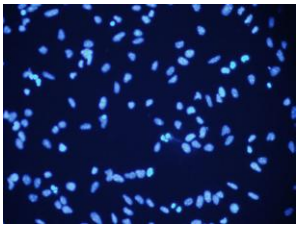
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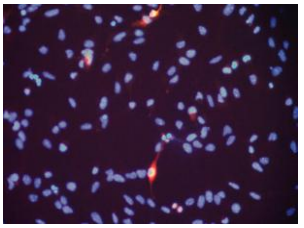
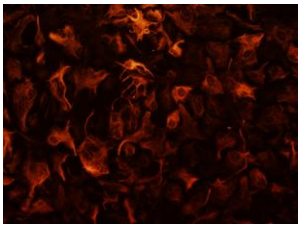

P4-merge-400×

The Immunofluorescence Picture of Induced Differentiation Neural Stem Cell

Astrocyte-specific Protein GFAP (Red), Neuron-specific

Protein Tubulin (Red), Hochest (Blue)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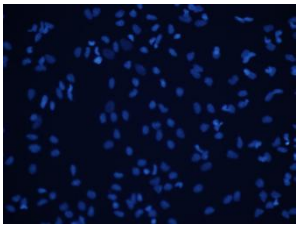
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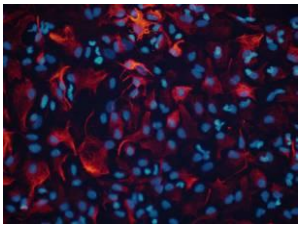
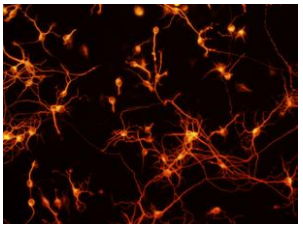
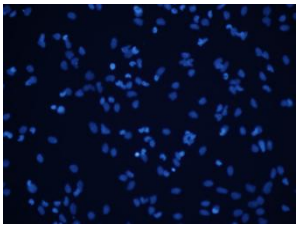

P4-merge-400×


P4-Tubulin-400×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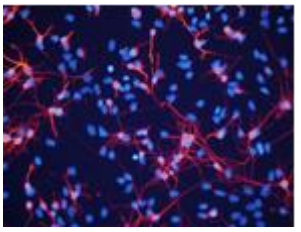
P4-merge-400×

Cryopreservation of Cells

Materials Required

- OriCell™ Protein-free Cryopreservation Medium For Neural Stem Cells (GUXNX-07031)
- OriCell™ NCR Protein-free Cryopreservation Medium For Neural Stem Cells (GUXNX-07021)

Note: OriCell™ Protein-free Cryopreservation Medium For Neural Stem Cells is a protein-free cryopreservation solution dedicated to neural stem cells. It is a ready-to-use cryopreservation solution with a clear chemical cost. It can be directly stored in the refrigerator at -80°C. There is no need to

freeze slowly step by step.

Steps

1. For cell collection, please refer to steps 1 to 6 in the passage operation of OriCell™ SD Rat Neural Stem Cells.
2. After the cells are collected, take a small amount of cell suspension and count with a hemocytometer to calculate the total number of cells.
3. After centrifuging the cell suspension at 160×g for 4 min, aspirate the supernatant.
4. Resuspend the cells with cryopreservation solution and adjust the cell density to 1×10^6 viable cells/mL (or expected cell density).
5. Divide the cells into cryopreservation tubes in proportion or quantity.

   Note: **In the absence of mature counting conditions, we recommend that the cells be divided into proportions for cryopreservation storage. Long-term storage under non-culture conditions will seriously affect the state of the cells. When counting, we recommend placing the cells in a refrigerator at 4°C to reduce cell metabolism and better maintain the cell state.**

6. If you choose OriCell™ Protein-free Cryopreservation Medium For Neural Stem Cells, put the cryopreservation tube into the pre-cooled freezing containers, and then put the freezing containers in the -80°C refrigerator. If you choose OriCell™ Protein-free Cryopreservation Medium For Neural Stem Cells, please disperse the cryopreservation tube directly into the refrigerator at -80°C.

   Note: **During the cryopreservation of cells, especially within 4 hours of the beginning of cryopreservation, the refrigerator door should not be opened, which will seriously affect the survival rate of cells.**

7. After 24 h, the cells were transferred to liquid nitrogen for long-term storage.

   Note: **Cells cannot be stored in a refrigerator at -80°C for a long time. We recommend that the storage time in the refrigerator at -80°C should not exceed 48h.**