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User Manual

OriCell™ Sprague-Dawley (SD) Rat Neural Stem Cells (NSCs)

Cat. No. RASNF-01001

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CONTENTS AND STORAGE

Product Name	Sprague-Dawley Neural Stem Cells
Catalog No.	RASNF-01001
Amount per Vial	1×10^6 Cells
Cryopreserved At	Second Passage
Storage Condition	Liquid Nitrogen

Caution: **Please handle this product as a potentially biohazardous material. This product contains Dimethyl Sulfoxide (DMSO), a hazardous material, in the freezing medium.**

PRODUCT INTRODUCTION

Neural Stem Cells (NSCs) are multipotent stem cells that can differentiate into a variety of cell types of the neural system, including neurons, astrocytes, oligodendrocytes and etc. NSCs have been isolated from various areas of the adult brain, and non-neurogenic areas, such as the spinal cord. They are capable of rebuilding functional neural circuits, thus offering a potential cure for brain tissue impairments. They have being widely studied in animal models for treating neural degenerative diseases, hereditary central nervous system diseases, stroke and spinal cord injuries.

Cyagen OriCell™ SD Rat Neural Stem Cells (NSCs) are derived from brain tissues of SD rat embryos (14.5 dpc), cultured as neurospheres, and cryopreserved at the second passage.

In addition, these cells have been tested for:

- Exogenous Factors: bacterial/fungal contamination, mycoplasma contamination, and endotoxin contamination.
- Characteristics: post-thaw viability, cell cycle, verification of undifferentiated state, and differentiation potential.

This product is intended for laboratory research use only. It is not intended for diagnostic, therapeutic, clinical, household, or any other applications.

CELL CHARACTERISTICS AND IDENTITY

- Strong capacity to expand. Can be passaged at least 3 times.

- Ability to differentiate into neurons, oligodendrocytes, and astrocytes
- Positive for nestin (> 75%), and negative for GFAP, Tubulin (< 10%).

PRODUCT APPLICATIONS

Neural stem cells have been widely studied both for developmental biology research and for therapeutic applications to the damaged brain. Transplantation of neural stem cells or their derivatives into a host brain is considered as potential treatment for neural degenerative disease, hereditary central nervous system diseases, stroke and spinal cord injuries.

GENERAL HANDLING PRINCIPLES

1. Aseptic handling of the product is necessary throughout.
2. Once the cells have been established, always freeze several vials of OriCell™ SD Rat NSCs as a backup.

Note: **The OriCell™ SD Rat NSCs can be frozen/thawed at least one times.**

3. For general maintenance of cells, we recommend the seeding density to be 1.0-2.0×10^5cells/mL.
4. For all studies, it is strongly recommended to use cells that are at, or under, an original passage number of 8.
5. When the neurospheres have a dark clump inside or ruffling on the outside of the neurosphere, it is recommended to split the cells.

Note: **We strongly recommend the use of OriCell™ culture media and other related reagents for optimal results.**

THAWING AND ESTABLISHING OriCell™ SD RAT NEURAL STEM CELLS (NSCs) (NEUROSPHERES CULTURE)

Materials Required:

- Phosphate-Buffered Saline (1×PBS) (Cat. No. PBS-10001)
- OriCell™ SD Rat Neural Stem Cells (Cat. No. RASNF-01001)
- OriCell™ Neural Stem Cell Growth Medium (Cat. No. GUXNX-90011)

Thawing and Establishing SD Rat NSCs

1. Pre-warm the OriCell™ NSC Growth Medium to 37°C.
2. Add 9 mL of OriCell™ NSC Growth Medium to a 15 mL conical tube.

3. Remove the cryovial of OriCell™ SD Rat NSCs from liquid nitrogen. Quickly thaw the vial in a 37°C water bath until the last crystal piece disappears, and finish the thawing procedure within 3 minutes. Be careful not to submerge the entire vial. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

Note: **Results will be less than optimal if the cells are thawed for more than 3 minutes.**

4. As soon as the cells are completely thawed, disinfect the outside of the cryovial with 70% v/v ethanol.
5. Use a pipette to transfer the cells to the conical tube containing containing OriCell™ NSC Growth Medium inside a biosafety cabinet. Be careful not to introduce any bubbles during the transfer process.
6. Rinse the vial with 1 mL of medium to reduce the loss of cell and then transfer this 1mL of cell suspension to the conical tube.
7. Gently mix the cell suspension by slowly pipetting up and down. Be careful not to introduce any bubbles.
8. Centrifuge the cell suspension at 250 x *g* for 5 minutes.
9. Carefully aspirate off as much of the supernatant as possible and add 3 mL of fresh OriCell™ NSC Growth Medium (pre-warmed to 37°C).
10. Gently re-suspend the cells in OriCell™ NSC Growth Medium.
11. Seed the cell suspension into a T25 flask and add a sufficient amount of OriCell™ NSC Growth Medium.
12. Incubate the cells at 37°C in a 5% CO_2 humidified incubator.

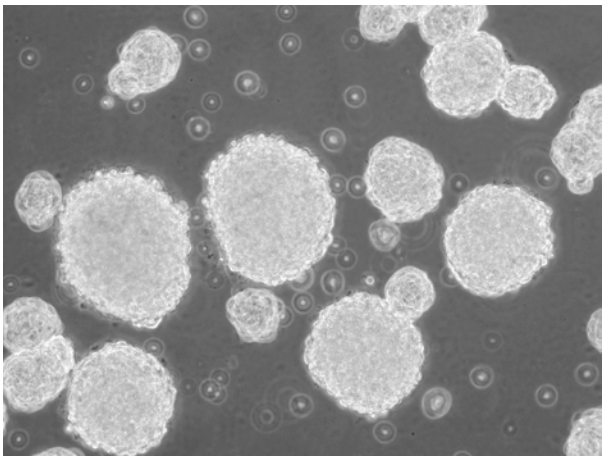

Fig.1 OriCell™ SD Rat Neural Stem Cells are established

PASSAGING OriCell™ SD RAT NSCs

Materials Required:

- Trypsin-Like Enzyme Solution
- Phosphate-Buffered Saline (1×PBS) (Cat. No. PBS-10001)
- OriCell™ SD Rat Neural Stem Cells (Cat. No. RASNF-01001)

- OriCell™ Neural Stem Cell Growth Medium (Cat. No. GUXNX-90011)

Passaging SD Rat NSCs

1. Pre-warm OriCell™ Neural Stem Cell Growth Medium, 1×PBS, Trypsin-Like Enzyme solution to 37°C.
2. Transfer the media containing the floating neurospheres to a 15 mL conical tube.
3. Centrifuge at 250 x *g* for 5 minutes.
4. Aspirate and discard all the supernatant and add 2mL of 1×PBS and 200µL of Trypsin-Like Enzyme to the conical tube and resuspend with a fire polished glass pipette.
5. Mechanically dissociate the neurospheres by gently pipetting up and down 8-10 times with a fire polished glass pipette, be careful not to introduce bubbles in the suspension.
6. Add 10 mL 1×PBS to the conical tube and mix well.
7. Centrifuge at 250 x *g* for 5 minutes.
8. Carefully aspirate off as much of the supernatant as possible.
9. Re-suspend the cells in 3 mL of OriCell™ Neural Stem Cell Growth Medium (pre-warmed to 37°C).
10. Plate cells into two or three T25 flasks and add sufficient OriCell™ Neural Stem Cell Growth Medium.
11. Incubate the cells at 37°C in a 5% CO_2 humidified incubator.

Additional Tips

Time to Split OriCell™ SD Rat Neural Stem Cells

When the neurospheres have a dark clump inside or ruffling on the outside of the neuro-sphere, it is recommended to split the cells. We typically split OriCell™ SD Rat Neural Stem Cells every two days.

OriCell™ SD RAT NSCs DIFFERENTIATION

Neural Stem Cells (NSCs) are maintained in serum-free culture medium supplemented with the mitogenes epidermal growth factor (EGF) and fibroblast growth factor 2 (FGF2). Removal of the mitogenes results in spontaneous differentiation of NSCs into neurons, astrocytes and oligondendroytes.

Besides spontaneous differentiation, NSCs can be directly differentiated into those lineages in defined conditions.

NSCs cultured in serum medium for about 7 days will lead to spontaneous differentiation into neurons (16±7%), astrocytes (75±7%) and oligondendroytes (5±3%).

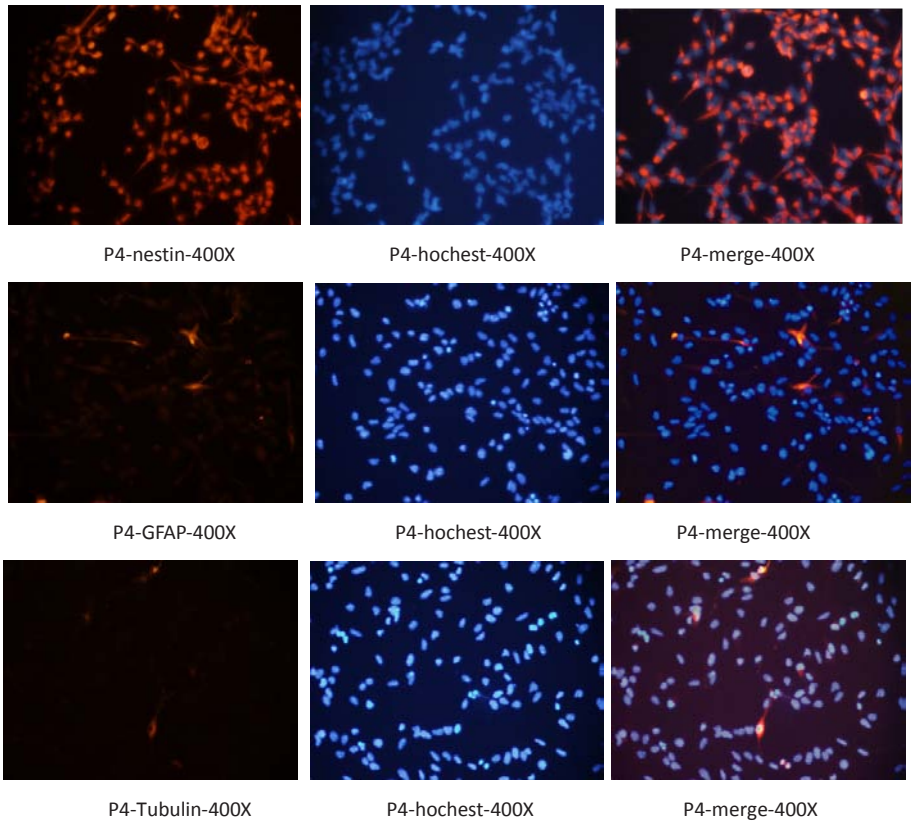

Fig.2 Immunofluorescence images (400x) of OriCell™ Neural Stem Cells stained for the undifferentiated NSC marker, nestin (red), hochest (blue), the astrocyte marker GFAP (negative), and the neuron marker Tubulin (negative).

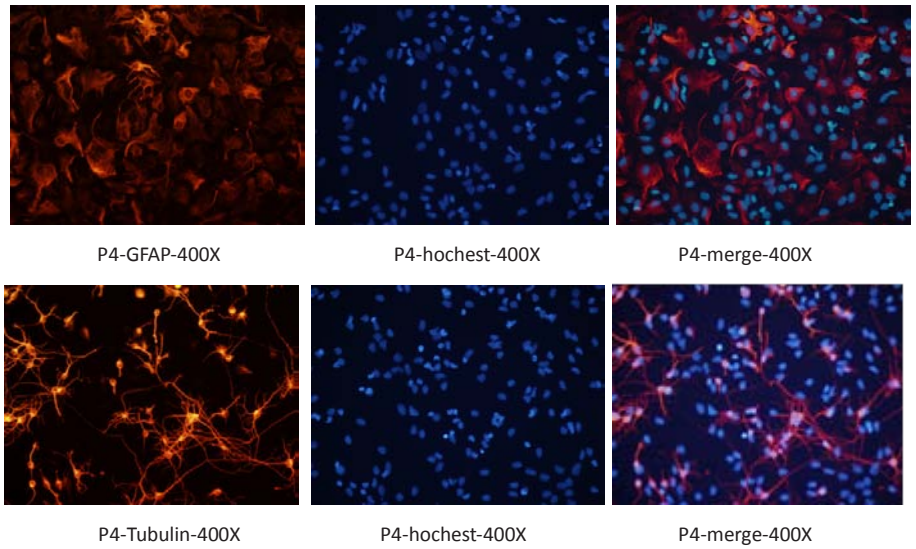

Fig.3 Immunofluorescence images (400X) of OriCell™ Neural Stem Cells stained for the astrocyte marker GFAP (red) and the neuron marker Tubulin (red).

CRYOPRESERVATION OF NSCs USING OriCell™ NSCs NCR PROTEIN-FREE CRYOPRESERVATION MEDIUM

Materials Required:

OriCell™ Neural Stem Cell NCR Protein-Free Cryopreservation Medium (Cat. No. GUXNX-07021)

OriCell™ Neural Stem Cell NCR Protein-free Cryopreservation Medium is a protein-free freezing medium for NSCs. It is formulated by defined chemical composition and can be used directly. The cells can be frozen directly in -80°C without step cooling down.

Cryopreservation

Note: **Change the culture medium with fresh growth medium 24 hours before freezing.**

1. Collect cells that are in the logarithmic growth phase. Perform a cell count to determine the viable cell density.
2. Centrifuge the cells for 3-5 minutes at 250 x *g* and 20°C. Remove and discard the supernatant using a pipette.
3. Resuspend the cell pellet in the OriCell™ NSCs NCR Protein-Free Cryopreservation Medium at a cell density of 10^5-10^6 cells/mL.
4. Dispense aliquots of the cell suspension into cryogenic storage vials that are properly labeled.
5. Place the vials directly in a -80°C freezer. After 24 hours, transfer the frozen vials to liquid nitrogen for long-term preservation.

APPENDIX

TROUBLESHOOTING

The table below lists some potential problems and solutions for culturing OriCell™ SD Rat NSCs.

Problem	Cause	Solution
Low cell recovery rate	The storage condition does not meet the requirements	Purchase a replacement, and store in liquid nitrogen for long-term preservation.
	Thawing of the cells takes too long	Thaw cells for no more than 3 minutes.
	Cells are incompletely recovered after thawing	After aspirating off medium, wash the tube with culture medium twice and transfer all of the cells to the dish.
	Cells are handled roughly	Care should be taken to avoid introducing bubbles during pipetting. Also avoid vortexing and high-speed centrifugation
	Medium is not pre-warmed	Warm medium to 37℃ before recovery.
Slow cell growth	Mycoplasma contamination.	Discard the cells in question and disinfect the laboratory environment before recovering the next batch of cells.
	Plating density is too low	Increase the plating density.
	Cells have been cultured for too long	Cells should be used up in 5-7 days after recovery
Cell aging	Inappropriate medium	Use Cyagen tailor-made culture media. If other media products are used, please perform validation to ensure compatibility.
	Dead cells are not removed promptly	Change the medium the next day after recovery to ensure removal of all dead cells.
	Cell Contamination	Discard the cells in question and disinfect the laboratory environment before recovering the next batch of cells.
	Plating density is too low	Some stem cells can secrete factors to support cell growth. Therefore, a certain degree of plating density must be maintained; otherwise, it will lead to cell proliferation slow down, and finally cell aging.

RELATED PRODUCTS

Product	Catalog Number
Trypsin-Like Enzyme	
Phosphate-Buffered Saline (1×PBS)	PBS-10001
OriCell™ SD Rat Neural Stem Cells	RASNF-01001
OriCell™ Neural Stem Cell Growth Medium	GUXNX-90011
OriCell™ Neural Stem Cell NCR Protein-Free Cryopreservation Medium	GUXNX-07021

REFENRENCES

Sally, Temple.(2001) The development of neural stem cells. Nature 414: 112-117.

Nigel L. Kennea, and Huseyin Mehmet. (2002) Neural stem cells. *The Journal of Pathology* 197: 536-550.

Rossella Galli, Angela Gritti, and Luca Bonfanti. (2003) Neural Stem Cells. *Circulation Research* 92: 598-608.

TECHNICAL SUPPORT:

Please visit the Cyagen website at www.cyagen.com for technical resources, additional product information, and special offers. You may also write, email, call, or fax to us at:

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Material Safety Data Sheets (MSDSs) are available upon request.

The Certificate of Analysis (CoA), which provides detailed quality control information for each product, is also available at the Cyagen website.