PrimaPure™

A division of Gene Therapy Systems, Inc.

Rat Neural Stem Cells (RNSC), Cryopreserved

Catalog #	Description/Content	Amount
SR82020A	RNSC, Adult	>500,000 cells
SR82020F	RNSC, Fetal	>500,000 cells
SR82020AK	RNSC, Adult, Complete System*	1 Kit
SR82020FK	RNSC, Fetal, Complete System*	1 Kit

*Each complete system contains an ampoule of cryopreserved RNSC (SR82020A or SR82020F), 250 ml of Rat Neural Stem Cell Growth Medium (SMR813250), and two non-tissue culture treated 10 cm plates. Rat Neural Stem Cell Differentiation Medium and the Poly-D-Lysine with Laminin Coating Solution are sold separately.

Related Products	Catalog #
Rat Neural Stem Cell Growth Medium, 250 ml	SMR813250
Rat Neural Stem Cell Differentiation Medium, 250 ml	SMRD813250
Poly-D-Lysine with Laminin Coating Solution, 2.5 ml	PR12725
NeuroFECT™ Transfection Reagent, 0.75 ml	T800075
GeneSilencer® siRNA Transfection Reagent, 200 reactions	T500750

Storage:	Store cryopreserved cell vial in liquid nitrogen immediately upon arrival. Store the growth medium at 4°C in the dark immediately upon arrival. Store the Subculture Reagent Kit at -20°C upon arrival and store the reagents at 4°C upon thawing.

INTRODUCTION

Rat Neural Stem Cells (RNSC) are isolated from cerebral striatum and mesencephalon of E18 fetal rat brains. These cells were maintained in undifferentiated proliferative state by culturing them as free floating neurospheres in serum-free medium optimized with growth factors. They are cryopreserved at second passage and can be cultured and propagated for 1- 2 passages[1] prior to induction of phenotypic differentiation. These stem cells are multipotent[2,3] and can be induced to differentiate into neurons, astrocytes and oligodendrocytes that make up the central nervous system (CNS). Neurospheres transplanted into intact brain can survive, expand and differentiate into mature neurons, astrocytes and oligodendrocytes precursors[4] in 3 weeks. The ability of neural stem cells to retain multi-lineage potential and proliferate extensively *in vitro* provides new avenues for the treatment of neural degenerative diseases and injuries.

MATERIALS AND METHODS

I. Preparation for Culturing

1. Make sure your Class II Biological Safety Cabinet, with HEPA filtered laminar airflow, is in proper working condition.
2. Clean the Biological Safety Cabinet with 70% alcohol to ensure it is sterile.
3. Turn the Biological Safety Cabinet blower on for 10 min. before cell culture work.
4. Make sure all serological pipettes, pipette tips, and reagent solutions are sterile.
5. Follow the standard sterilization technique and safety rules:
   a. Do not pipette with mouth.
   b. Always wear gloves and safety glasses when working with rat neural stem cells.
   c. Handle all cell culture work in a sterile hood.

II. Culturing RNSC

A. PREPARING CELL CULTURE FLASKS FOR CULTURING RNSC

1. Take the Rat Neural Stem Cell Growth Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
2. Pipette 9 ml of Rat Neural Stem Cell Growth Medium* into a 15 ml conical tube.
3. Pipette 15 ml of Rat Neural Stem Cell Growth Medium to a 10 cm petri dish*

***Do Not Use Tissue Culture Treated 10 cm Dishes**

B. THAWING AND PLATING RNSC

***Pre-wet the pipette and tips with medium to reduce cells sticking to the pipette and tips and avoid the loss of cells.**

1. Remove the cryopreserved vial of RNSC from the liquid nitrogen storage tank using proper protection for your eyes and hands.
2. Turn the vial cap a quarter turn to release any liquid nitrogen that may be trapped in the threads, then re-tighten the cap.
3. Thaw the cells quickly by placing the lower half of the vial in a 37°C water bath for 1 minute.
4. Take the vial out of the water bath and wipe dry.
5. Decontaminate the vial exterior with 70% alcohol in a sterile Biological Safety Cabinet.
6. Remove the vial cap carefully. Do not touch the rim of the cap or the vial.
7. Add 1 ml of Rat Neural Stem Cell Growth Medium drop by drop to the cryovial of thawed RNSC.
8. Transfer cell suspension with 1 ml aerosol tip to a 15 ml conical tube prepared in Section IIA Step 2.
9. Centrifuge at 400 x g for 5 minutes to pellet the cells.
10. Aspirate the supernatant from the tube without disturbing the cell pellet.
11. Flick the tip of the conical tube with your finger to loosen the cell pellet.
12. Resuspend the RNSC in 5 ml of Rat Neural Stem Cell Growth Medium by gently pipetting the neurospheres and dissociating

them into single cells.

13. Transfer 5 ml of dissociated RNSC into 10 cm petri dish prepared in Section IIA Step 3. RNSC should float in the Rat Neural Stem Cell Growth Medium and not attach to the dish.
14. Incubate RNSC culture in a 37°C, 5% CO_2 humidified incubator.
15. Examine the culture every day to make sure that most of RNSC are floating. RNSC will proliferate and form neurospheres in 2 to 3 days. If some of the neurospheres start to attach to the dish, pipette to shoot a stream of media to resuspend plated neurospheres.
16. Change half of the medium at day 2 or 3. Tilt the petri dish in an angle to let the neurospheres settle to the bottom of the dish and gently pipette out the consumed culture medium without disturbing the floating neurospheres.
17. Passage RNSC when neurospheres are 75 µm in diameter usually around day 3. Cells in the center will become hypoxic when the neurospheres grow to diameter exceeds 100 µm.

III. Passaging RNSC

1. Transfer the medium containing neurospheres to a conical tube with pipette.
2. Centrifuge at 100 x g for 5 minutes.
3. Remove supernatant, leaving behind approximately 200µl media.
4. Flick the tip of the conical tube with your finger to loosen the cell pellet.
5. Decontaminate a 200 µl Pipetman by wiping with alcohol and set the volume to 180µl.
6. Gently triturate the cell pellet 10-15 times with a pre-wet 200µl aerosol tip without introducing bubbles by expelling the cell suspension against the bottom side of the conical tube.
7. If some neurospheres remain undissociated, triturate more to maximum of 35 times.
8. Count viable cells using Trypan Blue exclusion (1:10 dilution).
9. Resuspend at 12,500 cell/ml in Rat Neural Stem Cell Growth Medium and plate 20 ml in a 10 cm petri dish.
10. Incubate RNSC culture in a 37°C, 5% CO_2 humidified incubator.
11. Examine the culture every day to make sure that most of RNSC are floating. RNSC will proliferate and form neurospheres in 2 to 3 days. If some of the neurospheres start to attach to the dish, pipette to shoot a stream of media to resuspend plated neurospheres.
12. Passage RNSC when neurospheres are 75 µm in diameter. Cell numbers usually double every passage.

IV. Differentiating RNSC

A. PREPARATION OF CULTUREWARE FOR DIFFERENTIATION.

1. Dispense Poly-D-Lysine with Laminin Coating Solution into tissue culture ware or chamber slide with the ratio of coating solution to surface area at 1 ml to 5 cm^2. If coverslips are to be used transfer cleaned* 18 mm diameter coverslips into each well of a 12-well plate and add 1.5 ml of Poly-D-Lysine with Laminin Coating Solution per well and transfer cleaned* 13 mm diameter coverslip into each well of the 24-well plate and add 1 ml of Poly-D-Lysine with Laminin Coating Solution per well.
   a. Incubate the tissue culture ware with coating solution for a minimum of 1 hour and glass surface with coating solution for a minimum of 3 hours at 37°C.
   b. Aspirate the coating solution.
   c. Wash the coated surface three times for 15 minutes per wash with sterile PBS.

* Coverslips should be soaked in ethanol, and each slip should be individually cleaned by wiping with a Kim wipe. Cleaned coverslips should be sterilized by autoclaving.

B. DIFFERENTIATING RNSC

1. Resuspend dissociated RNSC at 12,500/ml in Rat Neural Stem Cell Differentiation Medium.
2. Seed 1 ml into each well of pre-coated 8-well chamber slide or 2 ml into each well of pre-coated 4-well chamber slide or 7.5 ml of dissociated RNSC into a pre-coated T-25 flask.
3. Incubate RNSC culture in a 37°C, 5% CO_2 humidified incubator.
4. Check the culture daily and change half of the Rat Neural Stem Cell Differentiation Medium every other day.
5. Observe the differentiation of RNSC into matured neurons, astrocytes and oligodentrocytes with inverted microscope. It usually occurs in 10-12 days, and the differentiated neural cells can be examined by immunocytochemical staining.

REFERENCES

1. Svendsen, C.N., *et al*. (1997) *Brain Res. Dev*. **99**:253.
2. Reynolds, B.A., *et al.*, (1992) *J. Neuroscience* **12**(11): 4565.
3. Reynolds, B.A., *et al*. (1996) *Develop. Bio.* **175**: 1.
4. Karbanova, J., *et al*. (2004) *Biomed. Papers* **148**(2): 217

LICENCE:

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10190 Telesis Court. San Diego, CA 92121 • Toll Free (888) 428-0558 • U.S. & Canada (858) 457-1919 • www.genlantis.com
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