CELL
APPLICATIONS, INC.

General Instructions for Culturing

Rat Dorsal Root Ganglion Neurons (RDRGN)

Be sure to wear face protection mask and gloves when retrieving cryovials from the liquid nitrogen storage tank. The dramatic temperature change from the tank to the room could cause any trapped liquid nitrogen in the cryovials to burst and cause injury.

Open all the packages immediately upon arrival and examine each component for shipping damage. Notify Cell Applications, Inc. or your distributor immediately if there is any problem.

I. STORAGE

A. CRYOPRESERVED VIALS (R8820N-05)

Store the cryovials in a liquid nitrogen storage tank immediately upon arrival.

B. PLATING (R823P-10) AND CULTURE MEDIUM (R823-100)

Store the Plating Medium and Culture Medium at 4°C in the dark immediately upon arrival.

D. RAT NEURON COATING SOLUTION II (029-05)

Store at -20°C immediately upon arrival.
Store at 4°C after thawing.

II. PREPARATION FOR CULTURING

1. Make sure the 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 human cells even though all the strains have been tested negative for HIV, Hepatitis B and Hepatitis C.
   c. Handle all cell culture work in a sterile hood.

III. CULTURING RDRGN

A. COATING CELL CULTURE WARE FOR RDRGN

1. Thaw Neuron Coating Solution II at room temperature.
2. Pipette enough amount of Neuron Coating Solution II to the culture ware (for example, 0.5 ml in each well of a 24-well plate) to cover the whole culture surface.
3. Incubate the culture ware at 37°C for overnight.
4. Aspirate Neuron Coating Solution II from the culture ware.
5. Rinse the culture surface twice with sterile PBS prior to use to remove unbound Neuron Coating Solution II.

B. PREPARING FOR SEEDING RDRGN

1. Take the Rat Ganglion Neuron Plating Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
2. Equilibrate the Rat Ganglion Neuron Plating Medium in a 37°C, 5% CO_2 humidified incubator for 1 hour.

C. THAWING AND PLATING RDRGN

1. Remove the cryopreserved vial of RDRGN 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 and watch the vial closely during the thawing process.
4. Take the vial out of the water bath when only small amount of ice left in the vial. Do not let cells thaw completely.
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. Resuspend the cells in the vial by gently pipetting the cells 2 times with a 2 ml pipette. Be careful not to pipette too vigorously as to cause foaming.
8. Transfer the cell suspension from the vial into a 50 ml tube. Dropwise add 9.5 ml of equilibrated Rat Ganglion Neuron Plating Medium to the cells while swirling the tube to mix. Rinse the cryovial to recover all of the content. Collect the medium to the tube.
9. Gently mix the cell suspension in the 50 ml tube by pipetting and aliquot 2 ml into each well of the pre-coated 24-well plate.
10. Put the lid back to the 24-well plate and rock gently to evenly distribute the cells.
11. Place the 24-well plate in a 37ºC, 5% CO_2 humidified incubator. Do not disturb the culture for 6 hours after inoculation.
12. Carefully change to 1 ml of fresh Rat Ganglion Neuron Culture Medium after 6 hours to remove all traces of DMSO.
13. Refresh all Rat Ganglion Neuron Culture Medium on day 3 and 5. Afterward, change half of the medium every 3 to 4 days.
14. When cultured under the recommended conditions, cell death occurs during the first few days after plating. The remaining pure RDRGN arborize and form complex neurite network in one week.

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