CELL
APPLICATIONS, INC.

General Instructions for Culturing

Rat Spinal Cord Neurons (RSpN)

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

CRYOPRESERVED VIALS (R8814N-20 and R8814N-40)

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

II. RECOMMENDED MATERIALS FOR RSpN CULTURES (NOT PROVIDED)

1. Neuron Coating Solution I (027-05)
2. Rat Neuron Plating Medium (R817P-10)
3. Rat Neuron Culture Medium (R817-100)

III. 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.

IV. CULTURING RSpN

A. COATING CELL CULTURE WARE FOR RSpN

1. Thaw Neuron Coating Solution I at room temperature.
2. Pipette enough amount of Neuron Coating Solution I 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 37ºC for overnight.
4. Aspirate Neuron Coating Solution I from the culture ware.
5. Rinse the culture surface twice with sterile PBS prior to use to remove unbound Neuron Coating Solution I.

B. PREPARING FOR SEEDING RSpN

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

C. THAWING AND PLATING RSpN

1. Remove the cryopreserved vial of RSpN 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 equilibrated Rat 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. A seeding density of 100,000 cells per cm^2 or above is recommended.

Product	Recommended Plating
R8814N-20	10 wells of a 24-well plate
R8814N-40	20 wells of a 24-well plate

Cell Applications Inc (hereinafter CAI) warrants that its products are manufactured with the utmost care and stringent quality control procedures. However, if you should ever have a problem with the products, we will either replace the products, or in the case we cannot deliver the products, provide you with a refund. Such warranty is applicable only when CAI's cells are used in conjunction with CAI's medium and subculture reagents, and vice versa.

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. For best results, do not disturb the culture for 24 hours after inoculation.
12. Change to fresh Rat Neuron Culture Medium after 24 hours or overnight to remove all traces of DMSO. Two milliliters of medium is recommended for 24-well plate culture to minimize the detrimental effect of evaporation on the culture.
13. Change half of the Rat Neuron Culture Medium every three day. Refreshing whole volume is not recommended as neurons are sensitive to changes in culture conditions.

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