CELL
APPLICATIONS, INC.

General Instructions for Culturing

Rat Hippocampal Neurons (RHiN)

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 (R886N-10)

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

B. PRE-PLATED CELLS (R887N-)

1. Examine under a microscope to check if all the cells are attached to the bottom of the multiwell plate. If not, notify CAI or your distributor immediately.
2. Decontaminate the exterior of the multiwell plate with 70% alcohol.
3. Place the sealed multiwell plate in a 37°C, 5% CO_2 humidified incubator for 2 hours as shipped.
4. In a sterile Biological Safety Cabinet, remove the seal of the multiwell plate very slowly and carefully.
5. Carefully aspirate the Transport Medium to remain 2 ml in each well of the 24-well plate or 200 µl in each well of the 96-well plate.
6. Place the multiwell plate in a 37°C, 5% CO_2 humidified incubator.
7. Change half of the medium every three days.

C. CULTURE MEDIUM (817-50)

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

D. NEURON COATING SOLUTION (027-5)

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 RHiN

Neurons are sensitive to osmolality and pH changes. Use 2 ml of Neuron Culture Medium for each well. Do not use peripheral wells.
Neurons from one cryovial can seed 6 wells of 24-well plate

A. COATING CELL CULTURE WARE FOR RHiN

1. Thaw Neuron Coating Solution at room temperature.
2. Pipette enough amount of Neuron Coating Solution to the culture ware (for example, 0.5 ml in each well of a 24-well plate) to cover the whole culture surface. Only coat the center wells.
3. Incubate the culture ware at 37ºC for overnight or at least one hour.

B. PREPARING CELL CULTURE WARE FOR CULTURING RHiN

1. Aspirate Neuron Coating Solution from the culture ware.
2. Rinse the culture surface twice with sterile PBS prior to use to remove unbound Neuron Coating Solution.

3. Add 2 ml of sterile H_2O or PBS to the peripheral wells
4. Take the Rat Neuron Culture Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
5. Pipette 2 ml of Rat Neuron Culture Medium to the coated 24-well plate.
6. Equilibrate medium in the 24-well plate in a 37ºC, 5% CO_2 humidified incubator for 1 hour.

C. THAWING AND PLATING RHiN

1. Remove the cryopreserved vial of RHiN 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 5 times with a 2 ml pipette. Be careful not to pipette too vigorously as to cause foaming.
8. Pipette 160 µl* cell suspension from the vial into each well containing 2 ml* of Rat Neuron Culture Medium. A seeding density of 100,000 cells per cm^2 or above is recommended.
   *Medium to cell suspension ratio at least 10 to 1 is recommended.
9. Place the lid back to the 24-well plate and rock gently to evenly distribute the cells.
10. 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.
11. Change to fresh Rat Neuron Culture Medium after 24 hours or overnight to remove all traces of DMSO. 2 ml of Neuron Culture Medium is recommended for 24-well plate culture to minimize the detrimental effect of evaporation on the culture.
12. 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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