

General Instructions for Culturing

Rat Cortical Neurons (RCoN)

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 (R882N-20)

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

B. PRE-PLATED MULTIWELL PLATES (R883N-6w, -24w, -96w)

1. Examine under a microscope to check if all the cells are attached to the bottom of the multiwell plates. If not, notify CAI or your distributor immediately.
2. Decontaminate the exterior of the multiwell with 70% alcohol.
3. Place the multiwell in a 37°C, 5% CO₂ humidified incubator for 2 hours as shipped.
4. In a sterile Biological Safety Cabinet, remove the Transport Medium by aspiration. Add fresh Culture Medium.
5. Place the multiwell in a 37°C, 5% CO₂ humidified incubator.
6. Change medium every other day.

C. NEURON COATING SOLUTION I (027-05)

Store the Solution at 4°C in immediately upon arrival.

D. NEURON PLATING MEDIUM (R817P-20)

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

E. NEURON CULTURE MEDIUM (R817-100)

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

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 protective lab gear (lab coat, gloves, safety glasses, etc.) when working with cell cultures.
 - c. Handle all cell culture work in a sterile hood.

III. CULTURING RCoN

A. COATING CELL CULTURE WARE FOR RCoN

1. Take Neuron Coating Solution I from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
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. Coat 20 wells for each cryovial of R882N-40.
3. Incubate the culture ware at 37°C 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 PLATING MEDIUM FOR SEEDING RCoN

1. Take the Neuron Plating Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
2. Equilibrate 20 ml of Neuron Plating Medium in a 37°C, 5% CO₂ humidified incubator for 1 hr.

C. THAWING AND PLATING RCoN

1. Remove the cryopreserved vial of RCoN 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 1 mL pre-wetted aerosol tip set at 950 µl. 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 20 mL equilibrated Neuron Plating Medium prepared in Section IIIB Step 2 to 2E6 RCoN while swirling the tube to mix. Rinse the cryovial to recover all 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 cm² or above is recommended.
*R882N-20 can seed 10 wells of a 24-multiwell 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 37°C, 5% CO₂ humidified incubator. For best results, do not disturb the culture for 24 hours after inoculation.
12. Equilibrate 20 mL Neuron Culture Medium for 1 hour in 37°C, 5% CO₂ humidified incubator to be used in Step 13.
13. Change to fresh 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.
14. Change half of the Neuron Culture Medium every three day. Refreshing whole volume is not recommended as neurons are sensitive to changes in culture conditions.