CELL APPLICATIONS, INC.

General Instructions for Culturing

Mouse Striatal Neurons (MStN)

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 (M8812N-10)

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

B. PRE-PLATED CELLS (M8813N-)

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. PLATING (M817P-10) AND CULTURE MEDIUM (M817-100)

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

D. NEURON COATING SOLUTION I (027-05)

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 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 MStN

A. COATING CELL CULTURE WARE FOR MStN

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 to cover the whole culture surface. Refer to Table 1 for recommended coating conditions.

Culture vessel	Surface area	Coating solution	Plating medium	Cell number	Culture medium
6-well	10 cm^2	1 ml	8 ml	1,000,000	2.5 ml
12-well	4 cm^2	0.5 ml	3 ml	400,000	1 ml
24-well	2 cm^2	0.25 ml	2 ml	200,000	0.5 ml
48-well	1 cm^2	0.125 ml	1 ml	100,000	0.25 ml
96-well	0.3 cm^2	0.1 ml	0.25 ml	33,000	0.1 ml

Table 1 Recommended coating and seeding conditions.

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 FOR SEEDING MStN

1. Take the Mouse Neuron Plating Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
2. Equilibrate enough volume of Mouse Neuron Plating Medium in a 37°C, 5% CO_2 humidified incubator for 1 hr. Refer to Table 1 for recommended volume.

C. THAWING AND PLATING MStN

1. Remove the cryopreserved vial of MCoN 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 equilibrated Mouse Neuron Plating Medium prepared in Section IIIB Step 2 to MCoN 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 appropriate volume into each well of the pre-coated culture vessel. A seeding density of 100,000 cells per cm^2 or above is recommended. Refer to Table 1 for recommended seeding conditions.
10. Put the lid back to the culture vessel and rock gently to evenly distribute the cells.
11. Place the culture vessel in a 37°C, 5% CO_2 humidified incubator. For best results, do not disturb the culture for 24 hours after inoculation.
12. Equilibrate enough volume of Mouse Neuron Culture Medium for 1 hour in 37°C, 5% CO_2 humidified incubator to be used in Step 13. Refer to Table 1 for recommended volume.
13. Change to fresh Mouse Neuron Culture Medium after 24 hours or overnight to remove all traces of DMSO.
14. Change half of the Mouse Neuron Culture Medium every three days. Refreshing whole volume is not recommended as neurons are sensitive to changes in culture conditions.

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