CELL
APPLICATIONS, INC.

General Instructions for Culturing
Mouse Neural Stem Cells (MNSC)

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. MNSC TOTAL KIT STORAGE

A. CRYOPRESERVED VIALS (MS820-20)

MNSC were cryopreserved as dissociated cells in Freezing Medium (040-50).
Store the cryovials in a liquid nitrogen storage tank immediately upon arrival.

B. GROWTH MEDIUM (M813-250)

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

C. NEURAL STEM CELL DISSOCIATION SOLUTION (076-20) Store at -20°C immediately upon arrival.

D. 2 NON-TISSUE CULTURE 10 CM PETRI DISHES
For initial seeding of cells

II. MNSC DIFFERENTIATION REAGENTS

*** Not included in the MNSC Total Kit**

A. POLY-D-LYSINE WITH LAMININ COATING SOLUTION (127-25**)**

Store at 4°C.

B. MNSC DIFFERENTIATION MEDIUM

- Neuron Lineage (M813D-100N)
- Astrocyte Lineage (M813D-100A)
- Oligodentrocyte Lineage (M813D-100O)

Store 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 lab coat, gloves, and safety glasses.
   c. Handle all cell culture work in a sterile hood.

III. CULTURING MNSC

A. PREPARING FOR CULTURING MNSC

1. Take the Mouse Neural Stem Cell Growth Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood and warm up to room temperature.
2. Pipette 10 ml of Mouse Neural Stem Cell Growth Medium to a non-tissue culture 10 cm petri dish for seeding.

B. THAWING AND PLATING MNSC

* **Pre-wet the pipette and tips with medium to reduce cells sticking to the pipette and tips and avoid the loss of cells.**

1. Remove the cryopreserved vial of MNSC 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 pre-wetted, 1 ml aerosol pipette tip

set at 950 ul. Be careful not to pipette too vigorously as to cause foaming.

8. Transfer the cell suspension from the vial into a 15 ml conical tube. Dropwise add 9 ml of Mouse Neural Stem Cell Growth 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. Centrifuge at 200 x g for 5 minutes to pellet the cells.
10. Aspirate the supernatant from the tube without disturbing the cell pellet.
11. Flick the tip of the conical tube with your finger to loosen the cell pellet.
12. Resuspend the MNSC in 5 ml of Mouse Neural Stem Cell Growth Medium and gently pipette to mix well
13. Transfer 5 ml of MNSC into 10 cm petri dish prepared in Section IIIA Step 2. MNSC should float in the Neural Stem Cell Growth Medium and not attach to the dish.
14. Incubate MNSC culture in a 37ºC, 5% CO_2 humidified incubator.
15. Examine the culture every day to make sure that all MNSC are floating. MNSC will proliferate and form floating neurospheres in 2 to 3 days. If some of the neurospheres start to attach to the dish, pipette to shoot a stream of media to the attached neurospheres.
    *Do not let neurospheres attached to the dish, dislodge attached neurospheres several times a day is necessary
16. Change half of the medium every 2 or 3 days. Tilt the petri dish in an angle to let the neurospheres settle to the bottom of the dish and gently pipette out the consumed culture medium without disturbing the floating neurospheres.
17. Dissociate neurospheres when they reach 100-200 μm in diameter.

IV. DISSOCIATING MNSC

1. Take out Neural Stem Cell Dissociation Solution from refrigerator and warm up to room temperature.
2. Collect neurospheres in a 15 ml conical tube and spin down at 800 rpm for 3-5 minutes.
3. Flick the tip of the Cell pellet at the bottom of the conical tube may be loose. Carefully aspirate medium without disturbing the cell pellet.
4. conical tube with your finger to loosen the neurosphere pellet
5. Resuspend neurospheres in 3-5 ml of Neural Stem Cell Dissociation Solution.
   Optional: Add 30-50 μl of DNase I.
6. Incubate in 37ºC water bath for 2 minutes.
7. Add 10 ml Growth Medium.
8. Spin down at 1200 rpm for 5 minutes.
9. Carefully aspirate medium.
10. Resuspend neurospheres in 1-2 ml Growth Medium.
11. Gently triturate 10-15 times with a fire polished glass pipette to break down spheres.
12. If undigested clusters are present, let them settle by gravity. Transfer cell suspension to a new tube.
13. Add another 1-2 ml Growth Medium to the undigested clusters.
14. Gently triturate 10-15 times with fire polished pipette.
15. Combine with cell suspension in Step 12 and plate.

V. DIFFERENTIATING MNSC

A. PREPARATION OF CULTURE WARE FOR DIFFERENTIATION

1. Dispense Poly-D-Lysine with Laminin Coating Solution into tissue culture ware or chamber slide with the ratio of coating solution to surface area at 1 ml per 5 cm^2.
2. If cover slips are to be used for differentiation:
   a. Clean each cover slip by: soaking in ethanol overnight, wiping with Kimwipes, and sterilizing in an autoclave.
   b. Coat each cover slip in the well with Poly-D-Lysine/Laminin Coating Solution:
      12-well plate: 18 mm circle coverslip with 1.5 ml
      24-well plate: 12 mm circle coverslip with 1 ml
3. Coating Procedure
   a. Incubate the tissue culture ware with coating solution for a minimum 1 hour and glass surface with coating solution for a minimum of 3 hours at 37ºC.
   b. Aspirate the coating solution.
   c. Wash the coated surface three times for 15 minutes per wash with sterile PBS.

B. DIFFERENTIATION

1. Seeding density for differentiation is 50,000 cells per cm^2.
2. Resuspend dissociated MNSC in Neural Stem Cell Growth Medium as 100,000 cells per ml.
3. Seed the following cell suspension volumes to each well:
   12-well format: 2 ml per well
   24-well format: 1 ml per well
   96-well format: 165 ul per well
4. Incubate MNSC in Mouse Neural Stem Cell Growth Medium overnight in a 37ºC, 5% CO_2 humidified incubator.
5. Change to Differentiation Medium of your specific choice the next day.
6. Check the culture daily and change half of the Neural Stem Cell Differentiation Medium every other day.
7. Observe the differentiation of MNSC into matured neuronal cells of your choice with inverted microscope. Approximately 2 to 3 weeks are needed for differentiation to neurons and oligodendrocytes; approximately 7 to 10 days to differentiate to astrocytes.

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