Cryopreserved Human Microglia (BX-0900)

CONTENTS

- One vial of ≥2 million cryopreserved human microglia (in 500 µL)

Immediately transfer the vial of cells from dry ice to liquid nitrogen.

ADDITIONAL MATERIALS NEEDED

- PDL-Coated 96-Well Plates
- Microglia basal medium (see Table 1)
- Microglia culture medium (see Table 2)

Table 1. Microglia Basal Medium

Component	Amount
DMEM/F-12 (Gibco#1330-032)	500 mL
N2 (Gibco #17502048)	5 mL
B27 (Gibco #17504044)	5 mL
NEAA (Gibco #11140-050)	5 mL
Chemically Defined Lipid Mix (Gibco #11905-031)	2.5 mL
Glutamax (Gibco #35050-061)	2.5 mL
AA2P Solution (Sigma #A8960, 2.5g in 43ml of DMEM/F12)	550 µL
2-Mercaptoethanol (Sigma #M3148)	4.2 µL
Pen/Strep (Gibco #15140-122) (if desired)	5 mL

Recommendation: Mix well and filter medium with 0.22 µm filter system before use.

Table 2. Microglia Culture Medium

Component	Stock Conc.	Final Conc.
Microglia Basal Medium (see Table 1)	1X	1X
M-CSF (Peprotech #300-25)	100 µg/mL	20 ng/mL
IL-34 (Peprotech #200-34)	100 µg/ml	100 ng/mL
TGF-β1 (Peprotech #100-21C)	5 µg/mL	2 ng/mL

Recommendation: Please see manufacturer's direction to prepare and store stock solution of cytokines and growth factors. Prepare fresh before seeding and medium addition/change.

PROCEDURE

Day 0: Thawing and Seeding the Microglia

1. Before thawing vials, prepare microglia basal medium (Table 1) and microglia culture medium (Table 2), and allow the medium to equilibrate to room temperature prior to completion of the Day 0 protocol.
2. Remove the cryovial from the liquid nitrogen and immediately place in a 37°C water bath. To minimize contamination, avoid submerging the cap. Gently move the vial within the bath to increase the rate of thawing.
3. As soon as the last of the ice melts, which will take ~75-90 seconds, remove the vial from the water bath. Disinfect the vial by spraying it with 70% ethanol and transfer it to the cell culture hood.
4. Slowly add 500 µL of microglia basal medium (Table 1) to the vial at a rate of ~1 drop/s using a 1 mL pipette tip. This process should take about 30 seconds.
5. Gently transfer all contents (1 mL total) from the vial to a new sterile 15 mL conical tube.
6. To collect any residual cells, gently add another 1 mL of microglia basal medium (Table 1) to the vial and then transfer to the conical tube.
7. Slowly add an additional 3 mL of DMEM/F12 or microglia basal medium (Table 1) to the 15 mL conical tube using a 5 mL serological pipette. Gently swirl the conical tube while adding the medium. This process should take about 1 minute.
8. Centrifuge the cell suspension at 300xg for 5 min.
9. Aspirate supernatant and resuspend cell pellet in 1-2 mL of microglia culture medium (Table 2).
10. To count the cells, make sure the cell suspension is homogeneous by pipetting up and down twice with 1 mL pipette, and then take 10 µL from the cell suspension. Count the number of viable cells per mL with a hemocytometer using the trypan blue exclusion method to identify dead/viable cells.
11. Cells are then ready to seed. A seeding density of 20,000 cells/well (in 100 µL in 96-well format) is generally recommended, but the seeding density may vary based on intended use.
12. After seeding, do not immediately transfer the plate to the incubator. Leave the plate in the hood for 15 minutes to allow the cells to settle to the bottom of the well. After 15 minutes, very gently transfer the plate to a humidified incubator at 37°C with 5% CO_2.

Day 1: Medium Addition

1. Gently add 100 µL per well of freshly prepared microglia culture medium (Table 2) for 96-well format.
2. Optional: Some cells will not be attached to the bottom of the culture-ware on Day 1. To facilitate cell attachment, allow an additional day before medium change. Alternatively, collect the supernatant and spin down at 300xg for 5 min and re-plate with freshly prepared microglia culture medium to the original culture-ware.

Day 4: Medium Change

1. Gently remove 100 µL per well and replace with 100 µL per well of freshly prepared microglia culture medium (Table 2) for 96-well format. Repeat medium changes every 3 days for longer culture periods.