Cryopreserved Human Astrocytes (BX-0600 and BX-0650)

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- One vial of 2 million cryopreserved human astrocytes (200 µL)
- 200 µL Astrocyte Supplement at 1000X

Immediately transfer the vial of astrocytes from dry ice to liquid nitrogen. Transfer the vial of astrocyte supplement to a -20°C freezer. Once thawed, the supplements can be stored at 4°C for up to one month.

ADDITIONAL MATERIALS NEEDED

- DMEM/F12 Medium (Life Technologies #11330-032)
- Neurobasal Medium (Life Technologies #21103-049)
- N2 Supplement (Life Technologies #17502-048)
- GlutaMAX (Life Technologies #35050-061)
- Fetal Bovine Serum (Life Technologies #A3160601)
- PDL-Coated 96-Well Plates

PROCEDURE

Thawing and Seeding the Astrocytes

1. Gather the components for the Seeding Medium (see recipe below).
2. Each vial of cells requires approximately 20 mL of seeding medium. Working in a cell culture hood (biological safety cabinet), combine all components in a sterile 50 mL conical tube. Allow the medium to equilibrate to room temperature for 15 minutes. Do not warm the medium in a 37°C water bath.
3. Remove the cryovial from the liquid nitrogen and 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.
4. 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.
5. Slowly add 700 µL of seeding medium to the vial at a rate of ~1 drop/s using a 1 mL pipette tip. This process should take about 30 seconds.
6. Gently transfer all contents (1mL total) from the vial to a new sterile 50 mL conical tube.
7. To collect any residual cells, gently add another 1 mL of seeding medium to the vial and then transfer to the conical tube.
8. Slowly add an additional 3 mL of seeding medium to the 50 mL conical tube using a 10 mL serological pipette. Gently swirl the conical tube while adding the medium. This process should take about 1 minute.
9. To count the cells, gently swirl the conical tube again and remove 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.
10. The recommended seeding density is 20,000 viable cells/well for a 96-well plate (~60,000 viable cells/cm^2). Use the following equation to determine the volume of cell suspension needed for each 96-well plate: volume of cell suspension needed (mL) = (2.4x10^6 cells)/(viable cells per mL)

11. In a separate 50 mL conical tube, add the calculated volume of cell suspension needed, and then add enough medium to obtain a final volume of 12 mL. For example, if the volume of cell suspension needed is 2 mL, combine 2 mL of cell suspension with 10 mL of medium.
12. Mix completely and then plate 100 µL/well (20,000 cells/well) onto a PDL-coated 96-well plate using a multi-channel pipettor or liquid handler. Throughout the seeding process, be careful not to move or agitate the plate as this may lead to uneven attachment.
13. After seeding, do not immediately transfer the plate to the incubator. Leave it 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.
14. Note: the day the cells are plated is designated as Day 0.

Day 1 Medium Change

1. On Day 1 (24 hours after seeding), prepare fresh medium according to the Day 1 Medium composition below. Note that this medium is the same as the Seeding Medium but without Fetal Bovine Serum (see recipe below).
2. Very gently remove the entire 100 µL from each well and gently add 200 µL of the Day 1 Medium back to each well. Take care to ensure that the astrocytes do not dry out at any point in the medium changing process. We recommend changing one row or column at a time using a multichannel pipettor. Once complete, return the plate to the incubator.

Day 4 and Onward Medium Changes

1. Change half the medium (100 µL/well) twice weekly (on Day 4, 7, 11, 14, etc.). The medium for these changes is the same as above (see recipe below).
2. Gently withdraw 100ul/well, then add 100 µL/well fresh Medium to the entire plate.
3. The Astrocytes can be maintained viable and adherent in culture under the above conditions for at least 3 weeks post-seeding.

Media Compositions

		Component	Stock Conc.	Final Conc.	Volume
Seeding Medium	1	DMEM/F12 Medium	1X	0.5X	9.8 mL
	2	Neurobasal Medium	1X	0.5X	9.8 mL
	3	N2 Supplement	100X	1X	200 µL
	4	GlutaMAX	200 mM	0.5 mM	50 µL
	5	Astrocyte Supplement	1000X	1X	20 µL
	6	Fetal Bovine Serum	1X	1%	200ul

		Component	Stock Conc.	Final Conc.	Volume
Day 1-14 Medium	1	DMEM/F12 Medium	1X	0.5X	9.8 mL
	2	Neurobasal Medium	1X	0.5X	9.8 mL
	3	N2 Supplement	100X	1X	200 µL
	4	GlutaMAX	200 mM	0.5 mM	50 µL
	5	Astrocyte Supplement	1000X	1X	20 µL

		Component	Stock Conc.	Final Conc.	Volume
Day 18 Onward Medium	1	DMEM/F12 Medium	1X	0.5X	4.9 mL
	2	Neurobasal Medium	1X	0.5X	4.9 mL
	3	N2 Supplement	100X	1X	100 µL
	4	GlutaMAX	200 mM	0.5 mM	25 µL