BrainFast Neuronal Maturation Supplement

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- Two vials of 250 µL BrainFast Supplement

Immediately transfer supplement vials to a -20°C freezer. Once thawed, the supplements can be stored at 4°C for up to one week or aliquotted and returned to -20°C for up to six months.

ADDITIONAL MATERIALS NEEDED

- $5x10^6$ Primary Neurons or Early iPSC- or ESC-Derived Neurons
- DMEM/F12 Medium (Life Technologies #11330-032)
- Neurobasal Medium (Life Technologies #21103-049)
- B27 Supplement (Life Technologies #17504-044)
- N2 Supplement (Life Technologies #17502-048)
- GlutaMAX (Life Technologies #35050-061)
- Geltrex (Life Technologies #A1413201)
- BDNF (Peprotech #450-02)
- GDNF (Peprotech #450-10)
- TGF-β1 (Peprotech #100-21C)
- PDL-Coated 96-Well Plates

PROCEDURE

Plating the Neurons

1. Gather the components for the Seeding Medium according to the recipe below. Note that BDNF, GDNF, and TGF-β1 are supplied as lyophilized powders. Follow the manufacturer's instructions for reconstitution. We recommend creating stock solutions of 10 µg/mL for BDNF, 10 µg/mL for GDNF, and 1 µg/mL for TGF-β1.
2. Five million cells will require 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. Prepare cells either by thawing or by dissociation and spin-down. Dilute cells to approximately 1 million cells per mL for a total of 5 mL in a 50 mL conical tube.
4. To count the cells, gently swirl the conical tube 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 viable, dead, and total cells.
5. The recommended seeding density is 10,000-20,000 viable cells/well for a 96-well plate (~30,000-60,000 viable cells/cm^2). For 10,000 cells/well, use the following equation to determine the volume of cell suspension needed for each 96-well plate: volume of cell suspension needed (mL) = ($1.2x10^6$ cells)/(viable cells per mL).
6. 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.

7. Mix completely and then plate 100 µL/well (10,000 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.
8. 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.
9. 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 except for the addition of Geltrex.
2. To prepare the Geltrex, add cold DMEM/F12 directly to an aliquot of frozen Geltrex to yield a 1:10 dilution. For example, if aliquots of Geltrex have a volume of 100 µL, add 900 µL of cold DEME/F12. Immediately place this mixture at 4°C to allow the Geltrex to thaw and dissolve.
3. Very gently remove the entire 100 µL from each well and gently add 100 µL of the Day 1 Medium to each well. Take care to ensure that the neurons 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 Medium Addition

1. On Day 4 (96 hours after seeding), prepare fresh medium according to the Day 4 Medium composition below. Note that the Day 4 medium composition is the same as the Seeding composition.
2. Gently add 100 µL/well to the entire plate such that the total is now 100 µL/well.
3. Thereafter, change half the medium twice weekly (on Day 7, 11, 14, 18, etc.). The medium for these changes is the same as above excluding the supplements and Geltrex. Prepare this medium according to the Day 7 Onward Medium composition below.
4. The neurons mature rapidly can be maintained viable and adherent in culture for at least one month post-seeding.

Media Compositions

		Component	Stock Conc.	Final Conc.	Volume
Seeding/Day 4 Medium	1	DMEM/F12 Medium	1X	0.5X	9.6 mL
	2	Neurobasal Medium	1X	0.5X	9.6 mL
	3	B27 Supplement	50X	1X	400 µL
	4	N2 Supplement	100X	1X	200 µL
	5	GlutaMAX	200 mM	0.5 mM	50 µL
	6	BDNF	10 µg/mL	10 ng/mL	20 µL
	7	GDNF	10 µg/mL	10 ng/mL	20 µL
	8	TGF-β1	1 µg/mL	1 ng/mL	20 µL
	9	BrainFast Supplement	1000X	1X	20 µL

		Component	Stock Conc.	Final Conc.	Volume
Day 1 Medium	1	DMEM/F12 Medium	1X	0.5X	9.5 mL
	2	Neurobasal Medium	1X	0.5X	9.5 mL
	3	B27 Supplement	50X	1X	400 µL
	4	N2 Supplement	100X	1X	200 µL
	5	GlutaMAX	200 mM	0.5 mM	50 µL
	6	BDNF	10 µg/mL	10 ng/mL	20 µL
	7	GDNF	10 µg/mL	10 ng/mL	20 µL
	8	TGF-β1	1 µg/mL	1 ng/mL	20 µL
	9	BrainFast Supplement	1000X	1X	20 µL
	10	Geltrex	15 mg/mL	15 µg /mL	200 µL (of 1:10)

		Component	Stock Conc.	Final Conc.	Volume
Day 7 Onward Medium	1	DMEM/F12 Medium	1X	0.5X	9.6 mL
	2	Neurobasal Medium	1X	0.5X	9.6 mL
	3	B27 Supplement	50X	1X	400 µL
	4	N2 Supplement	100X	1X	200 µL
	5	GlutaMAX	200 mM	0.5 mM	50 µL
	6	BDNF	10 µg/mL	10 ng/mL	20 µL
	7	GDNF	10 µg/mL	10 ng/mL	20 µL
	8	TGF-β1	1 µg/mL	1 ng/mL	20 µL