24-well MEA GABAergic Neuron Assay (BX-0400, BX-0450, or BX-0700)

CONTENTS

- One vial of 5 million cryopreserved human neurons (500 µL)
- BrainFast GABA (BrainXell # BX-2400) (Formerly known as Neuron Seeding Supplement)
- BrainFast D4 (BrainXell # BX-2040) (Formerly known as Neuron Day 4 Supplement)
- BrainFast SK (BrainXell # BX-2020) (Formerly known as Supplement K)

ADDITIONAL MATERIALS NEEDED

- 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)
- 24-Well CytoView MEA Plates (Axion Biosystems # M384-tMEA-24W)
- BrainPhys Medium (STEMCELL Technologies # 05790)

EQUIPMENT NEEDED

- Maestro Edge (Axion Biosystems)

CELL CULTURE PROCEDURE

Thawing and Seeding the Neurons

1. Gather the components for the Seeding Medium according to the recipe below. Note that BDNF, GDNF, and TGF-β1 are 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. Working in a cell culture hood (biological safety cabinet), combine all components in an appropriately sized sterile container. For preparation of 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 DMEM/F12. Immediately place this mixture at 4°C to allow the Geltrex to thaw and dissolve before adding the appropriate amount to the Seeding Medium. Allow the Seeding Medium to equilibrate to room temperature for at least 15 minutes. Do not warm the medium in a 37°C water bath.
3. Remove a 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 ~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 500 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 per vial.
6. Gently transfer the contents (~1mL total) from the vial to new sterile 15 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 conical tube using a 5 mL serological pipette. Gently swirl the conical tube while adding the medium. This process should take about 1 minute.
9. Remove 10 µL from the cell suspension and count the number of viable cells per mL with a hemocytometer using the trypan blue exclusion method to identify dead/viable cells. Perform counting while tube is being centrifuged during step 10.
10. The recommended seeding density is 80,000 – 120,000 viable neurons/well. In order to cover one 24-well plate, calculate the volumes needed to add 2.4 – 3.6x10^6 neurons.
11. The cells must be concentrated to achieve a neuron concentration of 2,000 – 3,000 neurons/µL. Centrifuge the neurons at 1000 RPM (160xg) for 5 minutes and then carefully remove the liquid above the cell pellet. Once supernatant has been removed, add 500 µL of seeding media.
12. Mix neurons and seeding media gently by pipette until homogeneous and plate 40 µL in the center of each well onto a PDL-coated 24-well MEA plate, one well at a time.

Example Calculation for a desired concentration of 80,000 neurons/well:
24 well x 40 µL x 125% = 1,200 µL of total plating volume
Resuspended neuron conc. = $9.78x10^6$ viable cells/mL in 500 µL ($4.89x10^6$ viable neurons)
2,000 neurons/µL: (80,000 neurons x 24 wells x 125%) ÷ $9.78x10^6$ viable neurons/mL = 245 µL of resuspended neuron solution = 2,400,000 neurons (i.e. 80,000 neurons/well)
1,200 µL total volume – 245 µL neurons = 955 µL seeding media

13. Transfer the plate to a humidified incubator at 37°C with 5% CO_2 for 35 minutes.
14. After 35 minutes incubation remove plate from incubator and place in hood. Gently add 460 µL of seeding medium to each well, filling the bottom of each well while avoiding direct washing of the cells. Transfer the plate to a humidified incubator at 37°C with 5% CO_2. Day of cell plating is designated as Day 0.

*Note: Entire thawing and plating process should not exceed 2 hours, post-thaw viability and overall cell health will be severely impacted and lead to an unsuccessful culture.

Day 4 Medium Addition

1. On Day 4 (96 hours after seeding), prepare fresh Day 4 Medium (see recipe below).
2. Gently add 500 µL/well to the entire plate bringing the total volume in each well to 1 mL.

Day 7 and Onward Medium Changes

1. Change half the medium (500 µL/well) with Maintenance Medium every 3-4 days starting on Day 7 (ex: Day 7, 11, 14, 18, etc.) if pH indicator of media is changing more quickly, change media more regularly.

Media Compositions

		Component	Stock Conc.	Final Conc.	1 Plate Volume	2 Plate Volume	5 Plate Volume
Seeding Medium	1	DMEM/F12 Medium	1X	0.5X	9.5 mL	19 mL	47.5 mL
	2	Neurobasal Medium	1X	0.5X	9.5 mL	19 mL	47.5 mL
	3	B27 Supplement	50X	1X	400 µL	800 µL	2 mL
	4	N2 Supplement	100X	1X	200 µL	400 µL	1 mL
	5	GlutaMAX	200 mM	0.5 mM	50 µL	100 µL	250 µL
	6	BDNF	10 µg/mL	10 ng/mL	20 µL	40 µL	100 µL
	7	GDNF	10 µg/mL	10 ng/mL	20 µL	40 µL	100 µL
	8	TGF-β1	1 µg/mL	1 ng/mL	20 µL	40 µL	100 µL
	9	Geltrex	15 mg/mL	15 µg/mL	200 µL (of 1:10)	400 µL (of 1:10)	1 mL (of 1:10)
	10	BrainFast GABA Supplement	1000X	1X	20 µL	40 µL	100 µL
	11	BrainFast SK Supplement	1000X	0.5X	10 µL	20 µL	50 µL

		Component	Stock Conc.	Final Conc.	1 Plate Volume	2 Plate Volume	5 Plate Volume
Day 4 Medium	1	DMEM/F12 Medium	1X	0.25X	4.8 mL	9.6 mL	20 mL
	2	Neurobasal Medium	1X	0.25X	4.8 mL	9.6 mL	20 mL
	3	BrainPhys Medium	1X	0.5X	9.6 mL	19.2 mL	48 mL
	4	B27 Supplement	50X	1X	400 µL	800 µL	2 mL
	5	N2 Supplement	100X	1X	200 µL	400 µL	1 mL
	6	GlutaMAX	200 mM	0.5 mM	50 µL	100 µL	250 µL
	7	BDNF	10 µg/mL	10 ng/mL	20 µL	40 µL	100 µL
	8	GDNF	10 µg/mL	10 ng/mL	20 µL	40 µL	100 µL
	9	TGF-β1	1 µg/mL	1 ng/mL	20 µL	40 µL	100 µL
	10	BrainFast D4 Supplement	1000X	1X	20 µL	40 µL	100 µL
	11	BrainFast SK Supplement	1000X	0.5X	10 µL	20 µL	50 µL

		Component	Stock Conc.	Final Conc.	1 Plate Volume	2 Plate Volume	5 Plate Volume
Maintenance Medium	1	BrainPhys	1X	0.5X	19.3 mL	38.6 mL	96.5 mL
	2	B27 Supplement	50X	1X	400 µL	800 µL	2 mL
	3	N2 Supplement	100X	1X	200 µL	400 µL	1 mL
	4	GlutaMAX	200 mM	0.5 mM	50 µL	100 µL	250 µL
	5	BDNF	10 µg/mL	10 ng/mL	20 µL	40 µL	100 µL
	6	GDNF	10 µg/mL	10 ng/mL	20 µL	40 µL	100 µL
	7	TGF-β1	1 µg/mL	1 ng/mL	20 µL	40 µL	100 µL

MEA ASSAY

Neural activity should be present to some degree by DIV 12 and will plateau around DIV 21. For each recording, transfer the plate from the incubator to a warmed up (steady 37°C, 5% CO_2) the Maestro Edge. Allow 10-15 minutes for the plate to equilibrate (37°C, 5% CO_2) and then record for 10 minutes or desired length of time. After recording, return the plate to the incubator.