



24-well MEA Glutamatergic Neuron Assay (BX-0300)

CONTENTS

- One vial of 5 million cryopreserved human neurons (500 μ L)
- Neuron Seeding Supplement at 1000X
- Neuron Day 4 Supplement at 1000X
- Supplement K at 1000X

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- β 1 (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- β 1 are lyophilized powders. Follow the manufacturer's instructions for reconstitution. We recommend creating stock solutions of 10 μ g/mL for BDNF, 10 μ g/mL for GDNF, and 1 μ g/mL for TGF- β 1.
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 μ L, add 900 μ L 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 (~ 1 mL 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 $2.4 - 3.6 \times 10^6$ 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:

$24 \text{ well} \times 40 \mu\text{L} \times 125\% = 1,200 \mu\text{L}$ of total plating volume

Resuspended neuron conc. = 9.78×10^6 viable cells/mL in 500 μL (4.89×10^6 viable neurons)

2,000 neurons/ μL : $(80,000 \text{ neurons} \times 24 \text{ wells} \times 125\%) \div 9.78 \times 10^6 \text{ viable neurons/mL} =$

245 μL of resuspended neuron solution = 2,400,000 neurons (i.e. 80,000 neurons/well)

$1,200 \mu\text{L}$ 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	Seeding Supplement	1000X	1X	20 µL	40 µL	100 µL
	11	Supplement K	1000X	1X	20 µL	40 µL	100 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	Day 4 Supplement	1000X	1X	20 µL	40 µL	100 µL
	11	Supplement K	1000X	1X	20 µL	40 µL	100 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% CO₂) the Maestro Edge. Allow 10-15 minutes for the plate to equilibrate (37°C, 5% CO₂) and then record for 10 minutes or desired length of time. After recording, return the plate to the incubator.