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Protocol status: Working
We use this protocol and it's working

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Neuronal co-culture

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ABSTRACT

This protocol describes the co-culturing of iPSC-derived dopaminergic (DA) neurons and iPSC-derived medium spiny neurons (MSNs) in a microfluidic compartmentalization device.

ATTACHMENTS

Rafiq_co-culture.docx

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MATERIALS

Preparation of device for seeding cells:

Prepare enough amount of NB/B27 medium.

1. For 500 mL

- NB/B27 medium (see Method section of paper), add:

	A	B
	Neurobasal medium	484 mL
	B27 supplement without vitamin A	10 mL
	GlutaMAX	5 mL
	Penicillin-Streptomycin	1 mL

Storage: NB/B27 medium can be stored for 5 days at 4 °C or for up to one month at -20 °C .

- Warm NB/B27 medium at 37 °C .
- Make NB/B27 complete medium by adding:

	A	B
	BDNF	20 ng/ml
	Ascorbic acid	0.2 mM
	GDNF	20 ng/ml
	db-cAMP	0.5 mM
	TGFβ3	1 ng/ml
	DAPT	10 uM
	Y-27632	10 uM

Neuronal co-culture device set-up

1

Note

The OMEGA4 device has 2 pairs of interconnected chambers, where each pair of chambers is joined via a series of microfluidic channels.

Coat chambers with 200 µL per well with 0.1 undetermined Poly-L-Ornithine (PLO) in PBS.

2 Incubate plates overnight at 37 °C .

3 Wash the chambers thrice with PBS.

4 Coat chambers with 200 µL per well 10 undetermined Laminin plus 2 undetermined fibronectin, both diluted in PBS.

5 Incubate plates overnight at 37 °C . Do not store coated plates. Proceed with preparation of plates for seeding cells.

Preparation of device for seeding cells

15m

6 Prepare enough amount of NB/B27 medium.

1. For 500 mL

- NB/B27 medium (see Method section of paper), add:

	A	B
	Neurobasal medium	484 mL
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	DAPT	10 uM
	Y-27632	10 uM

7 Discard coating reagents and add 200 µL per well of NB/B27 complete medium. 15m

Keep the plate at 37 °C for 00:15:00 before seeding cells.

8 Replate cultured iPSC-derived dopaminergic neurons (day 30, see Method section of paper) on one side of the two-chamber microfluidic compartmentalization device (OMEGA4, eNuvio) at a cell concentration of 3×10^5. Only the axons of DA neurons can migrate through the microfluidic channels connected to the adjacent chamber.

9 Feed neurons with fresh NB/B27 media every 3 days. Add 10 undetermined Laminin to NB/B27 media every 10 days before feeding the neurons.

10 After an additional 25 days in the co-culture device, thaw frozen iPSC-derived medium spiny neurons (MSN) from BrainXell and plate on the other half of the device (where only the axons of DA neurons are present) at a cell concentration of $3x10^5$ cells.

11 Fix the DA-MSN co-cultures till 7-10 days later for immunofluorescence (see Method section of paper).