**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.

**Keywords**

Co-culture, microfluidic devices, dopaminergic neurons, MSNs

1. **Neuronal co-culture device set-up**

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

1.Coat chambers with 200 μL per well with 0.1 mg/ml 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 uL per well 10 μg/mL Laminin plus 2 μg/mL

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.

1. **Preparation of device for seeding cells:**

Prepare enough amount of NB/B27 medium.

For 500 mL

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

484 mL Neurobasal medium

10 mL B27 supplement without vitamin A

5 mL GlutaMAX

1 mL Penicillin-Streptomycin

Storage: NB/B27 medium can be stored for 5 days at 4 °C

or for up to one month at -20 °C

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Warm NB/B27 medium at 37 °C.

Make NB/B27 complete medium by adding:

20 ng/ml BDNF

0.2 mM Ascorbic acid

20 ng/ml GDNF

0.5 mM db-cAMP

1 ng/ml TGFβ3

10 uM DAPT

10 uM Y-27632

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

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

3. Cultured iPSC-derived dopaminergic neurons (day 30, see Method section of paper) were replated on one side of the two-chamber microfluidic compartmentalization device (OMEGA4, eNuvio) at a cell concentration of 3x105. Only the axons of DA neurons can migrate through the microfluidic channels connected to the adjacent chamber.

4. Feed neurons with fresh NB/B27 media every 3 days. Add 10 μg/mL Laminin to NB/B27 media every 10 days before feeding the neurons.

5. After an additional 25 days in the co-culture device, frozen iPSC-derived medium spiny neurons (MSN) from BrainXell were thawed and plated on the other half of the device (where only the axons of DA neurons are present) at a cell concentration of 3x105 cells.

5.The DA-MSN co-cultures were then fixed 7-10 days later for immunofluorescence (see Method section of paper).