Jun 26, 2023

Cortical, Striatal and Dopaminergic Neurons Cultures Protocol

DOI
dx.doi.org/10.17504/protocols.io.rm7vzxn48gx1/v1

Giulia Tombesi[1]

[1]Northwestern University, Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815

Giulia Tombesi
Northwestern University

DOI: https://dx.doi.org/10.17504/protocols.io.rm7vzxn48gx1/v1

Protocol Citation: Giulia Tombesi 2023. Cortical, Striatal and Dopaminergic Neurons Cultures Protocol. **protocols.io https://dx.doi.org/10.17504/protocols.io.rm7vzxn48gx1/v1**

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

Created: June 26, 2023

Last Modified: May 31, 2024

Protocol Integer ID: 84050

Keywords: ASAPCRN, co-cultures, primary neurons, striatal-cortical neuronal cultures, dopaminergic-cortical neuronal cultures, primary neuronal cultures from postnatal mice, dopaminergic neurons cultures protocol this protocol, dopaminergic neurons cultures protocol, pure cortical neurons culture, primary neuronal culture, cortical neuron, dopaminergic neuron, postnatal mice, neuron, striatal, postnatal, mice

Funders Acknowledgements:
Aligning Science Across Parkinson's [ASAP-020600] through the Michael J. Fox Foundation for Parkinson's Research (MJFF)
Grant ID: ASAP-020600

Abstract

This protocol describes how to obtain primary neuronal cultures from postnatal mice (P0-P1). It can be used to obtain pure cortical neurons cultures or stratal-cortical neurons and dopaminergic-cortical neurons co-cultures.

Materials

Papain Dissociation System from worthington biochemical corporation

Troubleshooting

Before start

Prepare the plates:

- Coat plates with poly-L-Lysine Overnight at room temperature or at least 02:00:00 at 37°C.
- Wash thoroughly with distilled water three times. Let it dry under the hood in the dark.

Prepare the following solutions:

- Dissection medium: 10mM HEPES pH7.3 , 0.1% D-glucose, 0.11mg/ml Sodium pyruvate in HBSS.
- DNase solution: one vial of DNAse in 500 µL of BME.
- Papain solution: one vial of Papain in 5 mL of BME plus 250 µL of DNase solution.
- Trypsin inhibitor solution: 15.5mg/ml in BME. Filter the solution.
- Stop solution: 250 µL of DNase solution, 600 µL of Trypsin inhibitor solution in 5.4 mL of BME.
- **10/10 solution:** Trypsin inhibitor 10ug/ml and BSA 10ug/ml in BME. Filter the solution.
- **Plating medium**: 1 mL B27 supplement, 125 µL L-Glutamine, 500 µL Pen/Strep, 500 µL N2 supplement in 50 mL Neurobasal.

Dissection

50m

1 Dissect the cortices, the striatum and the substantia nigra (SN) in cold dissection medium, carefully removing the meninges

Cells Dissociation

1h 5m

2 Snip the tissues into smaller pieces and place them in Papain solution. Use around 500 µL of Papain solution for 5 SN, around 1 mL of Papain solution for 5 striata and around 5 mL of Papain solution for 5-6 cortices.

3 Triturate 10 times with a 5ml-pipette

4 Incubate at 37°C for 00:40:00 and mix by inversion every 00:10:00

50m

Cells Dissociation

1h 5m

5 Triturate 10 times again with a 5ml-pipette

Cells Dissociation

1h 5m

6 Spin in swing bucket rotor at 1000rpm for 00:05:00

5m

7 Remove and discard the supernatant

8 Tap the tubes to move the pellet and add STOP solution. Add 1 mL for 5 SN, 1 mL for 5 striata and around 3 mL for 3 cortices.

9 Triturate 3 times with a 5ml-pipette

10 Incubate at room temperature for 00:10:00 10m

11 Take the supernatant and add to 10/10 solution drop-by-drop (use a volume of 10/10 solution that is equal to the supernatant volume)

12 Spin at 800rpm for 00:10:00 10m

Neuron Seeding

13 Discard the supernatant and resuspend in Neurobasal complete medium with AraC. Use 1 mL of medium to resuspend 5 striata and 3 cortices.

14 Count cortical and striatal cells with trypan blue

15 Plate 500000 cells/well in 24wells-plate.
For striatal-cortical co-culture plate a ratio of 1:2 striatum:cortex.
For SN-cortical co-culture plate one well for each SN and around 500000 cortical neurons/well and also add GDNF to the medium.

16 Replace half medium about every 7 days by collecting conditioned medium from cells and mixing with fresh complete medium in a ratio of 1:1.