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Protocol for mixed cortical striatal cell culture

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ABSTRACT

This protocol describes a optimised protocol for culturing cortical and striatal neurons from postnatal pup brains

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MATERIALS TEXT

Solutions Needed

- Poly-D-lysine

oStock: 200mg/ml

oDilute to 50µg/ml for coating

- AraC 1000x
- Basal Medium Eagle (dissection medium, nothing added, on ice)
- Basal Medium Eagle + supplement (water bath, used for initial incubation)
- Basal Medium – supplement + RFC serum
- Papain Enzyme

1 Add 500µl of poly-D-lysine solution to each well that will be used for plating

2 Allow to sit inside the hood for at least 30mins (this plate will stay under the hood until dissection is finished)

3 Set BME + supplement and BME+AraC serum in water bath

4 Add 1-2ml of BMS alone into 2 wells of a 24-well plate (not coated)

5 Add 4-5ml of BMS alone into 16mm culture dish

6 Place dish on ice and leave BME inside the hood

7 Dissect P0/P1 brains on ice in to dissection medium

8 Aspirate medium gently, add 1ml of enzyme and incubate for 20 minutes at 37 degrees

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9 Note: It is better to cut tissue sample using scalpel to allow more area for optimal enzymatic digestion

10 Wash 3X with 1ml of room temp BME medium without supplement to wash out the enzyme

11 Add 2ml of medium to well

12 Gently pipette 15 times and transfer to 15ml tube

13 Wait ~1min for large chunks to settle

14 Carefully remove supernatant and transfer to fresh 15ml tube

15 Centrifuge: 300g, 4°C, 4min

16 Remove media and resuspend in 1ml of media + serum

17 Remove poly-d-lysine from 24-well dishes

18 Add 500µl of medium + serum to each well

19 Count cells: 5x10^5 cells/well

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20 Plate 1:2 striatum:cortex

21 Incubate for 1hr then change media

22 Add GDNF to BME-(without serum; 1µl of GDNF in 2.5ml)

23 Incubate cells in a CO2 incubator

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