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Protocol for mixed cortical striatal cell culture

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Protocol status: Working

We use this protocol and it's working

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Abstract

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

Materials

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
- 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: 5×10^5 cells/well
- 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