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Primary culture cortical / hippocampal neurons E15-17 mouse - PFF testing

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

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Aligning Science Across Parkinsons

Abstract

This protocol is linked to the preparation of Pre Formed Fibrils by Ted Dawson's laboratory.

Tae-In Kam, Rong Chen, Ted Dawson . Production of alpha synuclein preformed fibrils (PFF). protocols.io https://protocols.io/view/production-of-alpha-synuclein-preformed-fibrils-pf-b39rqr56

This protocol for primary culture of cortical or hippocampal neurons is meant to test the toxicity of PFF *in vitro* before *in vivo* experiment/injections.

Guidelines

Volumes calculated for n=6 brains

Materials

DMEM (15ml)

- 1.5mL FBS heat inactivated
- 13.5mL DMEM medium

Maintenance media (50ml)

- 49ml Neurobasal™-A Medium
- 1 ml B27 serum free Gibco

Reagents

Fetal Bovin serum FBS (heat inactivated) **Thermo Fisher Scientific** **Catalog #**10082147

DMEM, High Glucose **Life Technologies** **Catalog #**11965-092

Poly-D-Lysine **MP Biomedicals** **Catalog #**02150175-CF

Neurobasal-A Medium **Contributed by users** **Catalog #**10888022

B27 supplement without retinoic acid (50x) **Gibco, ThermoFisher** **Catalog #**17504044

Coverslip **Electron Microscopy Sciences** **Catalog #**72229-01

Coating coverslip

4d 0h 30m

1 Reconstitute in 4 mL 4mL distilled sterile water.

2 Aliquot 100 µL and store at -20 °C .

3 Dilute the 100 µL aliquot in 25 mL PBS no Ca/mg.

4 Sterilize autoclaved coverslips under UV for 00:20:00 . 20m

5 Incubate the coverslips with Poly-D-Lysine (1 mL /12well plate) for 00:30:00 in incubator. 30m

6 Wash x3 with sterile water.

7 Dry out under the hood.

8 Add 500 µL Neurobasal A to the well and place in incubator.

Dissection/Culture

4d 0h 30m

9 Kill pregnant mice (E15-17) by CO_2 intoxication and cervical dislocation.

10 Dissect their embryos and collect in ice cold HBSS (no phenol red, no Ca/Mg).

11 Dissect and collect the brains in ice cold HBSS.

12 Dissect the cortex/hippocampus and separate and remove the soft membrane and blood vessels.

13 Collect all the cortices/hippocampus in 30 mL PBS On ice .

14 Transfer the cortices/hippocampus to a 15 ml tube containing 9 mL trypsin–EDTA (0.25%) and incubate at 37 °C for 00:15:00 , gently shake every 00:05:00 . 20m

15 Dissociate the cortices/hippocampus by triturating with a 10 mL serological pipette 10–15 times, or until no chunks are left.

16 Centrifuge the dissociated cortices/hippocampus at 1500 rpm for 00:05:00 . 5m

17 Resuspend the pellet in 10 mL DMEM high glucose with 10% FBS.

18 Triturate the cell suspension 10 times with a 1ml pipette.

19 Centrifuge at 1500 rpm for 00:05:00 5m

20 Resuspend the pellet in 10 mL Neurobasal-A Medium with 2% B-27 Supplement (50X).

21 Count the cells and plate in 12 well plate 450.000/well.

22 Change medium after 96:00:00 (4 days). 4d

PFF Incubation 1m

23 When neurons are at DIV7 proceed with PFF infection
N.B: If PFFs are added at DIV10 the aggregation is quicker.

24 Dilute 5mg/mL PFFs to 0.1mg/mL (20uL PFF+980uL PBS).

25 Sonicate at amplitude 20% for a total of 60 pulses (0.5 seconds on/off cycle). Pause briefly between every 10-12 pulses to prevent solution from heating up excessively and to avoid frothing.

26 Let it settle for 00:01:00 . 1m

27 Dilute to 1ug/mL (50uL PFFs+5mL neurobasal NB).

28 Filter in a 0.2um filter.

29 Replace completely the medium in well with NB+PFFs.

30 Incubate with PFFs for 10 days replacing half of the medium every 3 days.