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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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Funders Acknowledgements:

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-b39qr56>

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 **Catalog #10888022**

⊗ B27 supplement without retinoic acid (50x) **Gibco - Thermo Fisher Scientific 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 μ L and store at  -20 °C .
- 3 Dilute the  100 μ L 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 μ L Neurobasal A to the well and place in incubator.

Dissection/Culture

4d 0h 30m

- 9 Kill pregnant mice (E15-17) by CO₂ 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 .
- 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.

1m