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Culturing Primary Cortical Neurons

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This protocol is used to isolate and culture primary cortical neurons from mouse embryos at E14.5-E15.5.

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Day 1 1d 3h 16m

1 Coat culture dishes with 0.05mg/mL Poly-D-lysine overnight at 37°C. 1d

Day 2 3h 14m

2 Wash culture dishes with sterile water x2 then leave to dry. 1m

3 Sedate pregnant mouse with 120mg/kg Euthanyl and confirm sedation prior to proceeding with the next step. 2m
*The neurons must be plated within approximately 4 hours of sedation.

4 Soak pregnant mouse with 70% ethanol then dissect open the abdominal cavity. 1m

5 Isolate uterus with embryos and place in a tube of 1X PBS. 1m

6 In a laminal flow hood, dissect the brains of E14.5-15.5 embryos in Hank's Balanced Salt Solution (HBSS) and isolate each brain in 700uL of HBSS. 2h

7 Gently pipette up/down x10 to mix cortices then add 20uL trypsin for every embryo and incubate on a rotator at 37^{o}C for 20 minutes. 20m

8 Add 300uL Solution A per embryo and carefully pipette up/down x5 then centrifuge at 2500g for 5 minutes at 4^{o}C. 10m
Solution A: 2.75mL Neurobasal media (unsupplemented) + 150uL trypsin inhibitor + 100uL DNAse1

9 Remove supernatant and carefully resuspend pellet in 300uL Solution B per embryo by pipetting up/down x10. 5m
Solution B: 2.55mL Neurobasal media (unsupplemented) + 200uL trypsin inhibitor + 250uL DNAse1

10 Centrifuge at 2500g for 5 minutes at 4^{o}C then remove supernatant. 5m

11 Resuspend pellet in 1mL Neurobasal complete per embryo. 1m

11.1 Optional trypan blue cell exclusion assay: Mix 100uL trypan blue + 100uL 1X PBS + 20uL resuspended cells. Count with haemocytometer. 5m

12 Plate neurons on pre-coated dishes at desired confluency. 1m

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13 Change neurobasal medium at 4 days *in vitro.* 5m

14 Fix neurons at 7 days *in vitro* by removing media and replacing with 4% paraformaldehyde for 10 minutes at room temperature. 10m

15 Wash neurons with 1X PBS x2 to remove traces of paraformaldehyde then store in 1X PBS at 4^{o}C. 5m

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