

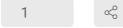


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Culturing Primary Cortical Neurons

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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

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.

*The neurons must be plated within approximately 4 hours of sedation.



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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°C for 20 minutes.	20m
8	Add 300uL Solution A per embryo and carefully pipette up/down x5 then centrifuge at 250 for 5 minutes at 4°C. Solution A: 2.75mL Neurobasal media (unsupplemented) + 150uL trypsin inhibitor + 100u DNAse1	
9	Remove supernatant and carefully resuspend pellet in 300uL Solution B per embryo by pipetting up/down x10. Solution B: 2.55mL Neurobasal media (unsupplemented) + 200uL trypsin inhibitor + 250u DNAse1	5m L
10	Centrifuge at 2500g for 5 minutes at 4°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 PBS + 20uL resuspended cells. Count with haemocytometer.	1 <mark>5</mark> m
12	Plate neurons on pre-coated dishes at desired confluency.	1m

- 14 Fix neurons at 7 days *in vitro* by removing media and replacing with 4% paraformaldehyde for 10 minutes at room temperature.
- 15 Wash neurons with 1X PBS x2 to remove traces of paraformal dehyde then store in 1X PBS at 4° C.