**Day 1**

*Prepare* cell culture plates by coating with Poly-D-Lysine and Mouse Laminin I.

**Day 2**

*Isolate* E17–18 rat embryos or P1–2 rat pups. *Decapitate.*

- **Cut** out C-shaped hippocampus from both hemispheres and *discard.*
- **Peel** off the meninges from both hemispheres and *discard.*
- **Separate** the cortical hemispheres and *discard* the brain stem.
- **Remove** the brains from the heads and place on ice in cold PBS.
For Embryonic Cortical Tissue


For Postnatal Cortical Tissue


Centrifuge @ 200 × g 5 minutes, room temperature

Centrifuge @ 200 × g 4–6 minutes, room temperature

For Embryonic Cortical Tissue

Centrifuge @ 200 × g 5 minutes, room temperature

Centrifuge @ 200 × g 5 minutes, room temperature

5 mL warmed Neuronal Base Media

10 mL warmed Neuronal Base Media

5 mL EBSS with 1 µg/mL Ovomucoid protease inhibitor

Incubate 20–30 minutes in a 37 ºC, 5% CO₂ humidified incubator

Warmed enzyme solution

15 mL conical tube


Wash cells twice. Centrifuge. Decant supernatant.

10 mL warmed Neuronal Base Media

15 mL conical tube

Fire-polished Pasteur pipette

Centrifuge @ 200 × g 4–6 minutes, room temperature

Centrifuge @ 200 × g 5 minutes, room temperature

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Resuspend cells in warmed Complete Cortical Neuron Culture Media. Count cells.

Reconstitute cortical neurons with warmed Complete Cortical Neuron Culture Media. Seed neurons onto Poly-D-Lysine/Laminin I-coated cell culture plates or µ-slides.

Culture cortical neurons for desired amount of time. Exchange media every 3–4 days.


Wash cells twice. Centrifuge. Decant supernatant.

10 mL warmed Neuronal Base Media

15 mL conical tube

Fire-polished Pasteur pipette

Centrifuge @ 200 × g 4–6 minutes, room temperature

Centrifuge @ 200 × g 5 minutes, room temperature

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