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Primary neuronal cultures

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ABSTRACT

This protocol describes the preparation of primary neuronal cultures from E15.5 CD-1 wild type mouse embryos. Experiments involving animal models must be performed in accordance with relevant institutional guidelines and regulations.

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1 Sacrifice pregnant female mice by cervical dislocation.

2 Remove the uterus from the abdominal cavity and place into a 10 cm sterile Petri dish on ice containing dissection medium, consisting of Hanks' balanced salt solution (HBSS) supplemented with 0.01 M HEPES, 0.01 M MgSO4 and 1% penicillin/streptomycin.

3 Isolate each embryo, decapitate the heads, remove the brains from the skull and immerse in ice-cold dissection medium.

4 Dissect cortical hemispheres, and remove meninges under a dissection microscope.

5 Collect the cortices in a 15 mL sterile tube and digest with 0.25% trypsin containing 1 mM ethylenediaminetetraacetic acid (EDTA) and 15 µL 0.1% DNAse I for 20 min at 37 °C.

6 Stop digestion by removing the supernatant and washing the tissue twice with Neurobasal medium (Invitrogen) containing 5% Fetal Bovine Serum.

7 Resuspend the tissue in 2 mL Neurobasal medium and triturate to achieve a single cell suspension.

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8 Spin cells at 130 x g, remove the supernatant, and resuspend the cell pellet in Neurobasal medium with 2% B-27 supplement (Invitrogen), 1% L-glutamine (Invitrogen) and 1% penicillin/streptomycin (Invitrogen).

9 Plate cells at desired density in dishes or coverslips coated with 1 mg/mL poly-D-lysine (Sigma) and 1 µg/mL laminin (Thermo Fisher Scientific).

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