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Primary Neuron Cultures

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We use this protocol and it's working

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Abstract

This protocol is for the isolation and preparation of primary cortical neuron cultures from the embryos of C57BL/6 mice.

Primary Neuron Cultures

1 Set up a breeder pair 18 days prior to harvest

2 When the female is close to giving birth coat a 6 well plate days before the dissection occurs and make the media

3 Prior to coating, sterilize coverslips by autoclaving

4 Using sterile forceps, transfer one pre-sterilized glass coverslip per well of a 6 well plate

5 Dispense 1-2 mL of Poly-L-Lysine (PLL) solution into each well containing a coverslip

6 Incubate for a minimum of 2 hours at 37°C or overnight at 4°C

7 Wash each well with 1 mL Tissue Grade Water two times

8 Add 2 mL of complete neurobasal media to each well of a 6 well plate

9 Prepare Sacrifice pregnant female by isoflurane/cervical dislocation

10 Surgically open peritoneum and remove embryonic sacks

11 Prepare 1950 µL of Enzyme Mix 1 for up to 400 mg of tissue (5 brains)

12 Pre-heat the mixture at 37°C for 10-15 min prior to use

13 Carefully open each sack and separate the embryo in a petri dish filled with cold HBSS without Ca2+ or Mg2+

14 Remove the head and the skullcap to excise the brain and get rid of any red blood cells

15 Separate the forebrain from the cerebellum and place the forebrain into a 15 mL conical tube containing 14 mL of HBSS (without Ca2+ or Mg2+)

16 Centrifuge at 300 x g for 2 min at room temperature and aspirate the supernatant

17 Add 1950 µL of enzyme mix 1

18 Incubate in a closed tube for 15 min at 37°C under slow, continuous rocking/rotation

19 Prepare 30 µL of enzyme mix 2 per eppendorf tube and add to the 15 mL conical and invert the tubes gently to mix

20 Dissociate tissue mechanically by passing through an 18 gauge needle 10 times

21 Incubate in a closed tube for 10 min at 37°C under slow, continuous rocking/rotation

22 Dissociate tissue again by passing through an 20 gauge needle 10 times

23 Incubate in a closed tube for 10 min at 37°C under slow, continuous rocking/rotation

24 Apply the cell suspension to a 40 m strainer placed on a 50 mL tube

25 Wash the filter with 10mL HBSS (with Ca2+ or Mg2+) (Sigma 55037C)

26 Centrifuge cell suspension at 300 x g for 10 min at room temperature and aspirate supernatant

27 Resuspend pellet in 2 mL of warm Neurobasal Media and count cells

28 Plate 500,000 cells per well in a 6 well plate

29 Change ONLY 50% of the media every three days with Neurobasal media without glutamic acid