

Cortical neuron culture

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Preparation:

- Prewarmed planting medium (DMEM/F-12 (cellgro) 500ml, FBS 50ml, N2 (Gibco 17502-048), P-S 5ml (cellgro, cat 30-002-CI)
- HBSS (without Ca²⁺ and Mg²⁺)
- 2.5mg/ml Trypsin (stock)
- 10mg/ml DNAase(stock)

Rats or Mice, E18 (or P0)

1. Euthanize the pregnant animal or pups.
2. Separate cortical in small dish with 1.8ml HBSS (without Mg⁺⁺ and Ca⁺⁺).
3. Mince tissue to small piece with spring scissor. Add 0.2 ml 2.5% Trypsin (10X) into 1.8ml HBSS, 37 C for 15 min.
4. Pipette up and down vigorously to dissociate cells. If large clumps are still obviously, remove the supernatant and add 0.2 ml 2.5% Trypsin and 1.8 ml HBSS again, incubate at 37 C for another 15 min. The separated supernatant should be added 2 ml plating medium to stop the activity of Trypsin. If cells are enough, no need to do the second digestion.
5. Add 1 ul DNAase(100X, 1% stock), dissociate cells by pipeting. Stop the activity of Trypsin by adding plating medium. Combine it with the previous supernatant, centrifuge at 1000 rpm (low rpm is OK) for 2-5 min.
6. Discard the supernatant, then resuspend the pellet with DMEM/F-12+10%FBS+N2 (plating medium), pipette up and down 6-8 times (pay attention not to make bubbles), make sure the cell clumps are dissociated sufficiently.
7. Pass cell solution through 40 um mesh (Cell strainer) to collect the dissociated cells.
8. Count cells by trypan blue staining (20 ul 0.08% trypan blue + 20 ul cell solution, incubate for 2-4 min at RT). Do not count the blue cells.
9. Plating cells at 5,000-20,000 density into one well of 12-well plate with poly-D-lysine coated coverslips. 1 ml medium per well (for low-density culture). Planting 4-6×10⁶ into 60mm dish, 3 ml medium per dish(for high-density culture).
10. Incubate 37 C, 5% CO₂ for 4 hours.

11. Thoroughly replaced plating medium with maintaining medium (Neural basal + B27 + 0.5 mM L-glutamine). Change 1/4-1/2 medium or add new medium every 3-4 days. The culture can be maintained for 4 weeks.