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Primary cortical mixed culture

Xiqun Chen¹, Qing Ye²

¹Department of Neurology Massachusetts General Hospital Harvard Medical School Charlestown MA 02 129 USA, Aligning Science Across Parkinson's (ASAP) Collaborative Research Network Chevy Chase MD 20815 USA;

²Department of Neurology Massachusetts General Hospital Harvard Medical School Charlestown MA 02 129 USA, Department of Neurology Longhua Hospital Shanghai University of Traditional Chinese Medicin e Shanghai 200032 China

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Pranay Srivastava

ABSTRACT

To obtain cortical culture, pregnant mice were anesthetized, embryos were dissected, and cortex was collected in PBS. Tissues were incubated in 0.25% trypsin–EDTA at 37°C for 15 min. Trypsinized tissue was transferred into a high-glucose DMEM/F12 medium supplemented with 10% FBS. After centrifugation (1500rpm, 5 min), the pellet was resuspended. Cells were plated onto poly(L-lysine)-coated 24-well plates at 10⁶cells per well and cultured in NB-A.

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- 1 For primary cortical mixed culture Use C57BL/6J mice at embryonic day 17
- 2 Anesthetized pregnant mice (1% sodium pentobarbital, 80mg/kg), dissect their embryos and collect the cortex.

(Separate and remove the soft membrane and blood vessels, rinse the cerebral cortex in PBS, and use the ophthalmic scissor to cut pieces of the cortex)

3 Collect the cortices in PBS in a 50 ml tube on ice (The 50 ml tube contains 30 ml of PBS) § On ice □5 mL) § On ice

- 4 Transfer the cortices to 15 ml tubes containing 1.5 ml trypsin-EDTA (0.25%) and incubate it at 37 °C for © 00:15:00 Dissociate the cortices by triturating with a 10 mL serological pipette 10 15 times
- 5 Centrifuge the dissociated cortices (\$1500 rpm , $\,\textcircled{\$}\textbf{00:05:00}$) and resuspend the pellet in $\overset{5m}{10ml}$

⋈ DMEM, high glucose Thermo Fisher

Scientific Catalog #11965092

medium supplemented with 10%

Fisher Catalog #10082147

6 Seed the cells onto poly(L-lysine)-coated 24-well plates at a cell density of 1x10⁶ cells/well containing specialized

⊠ Neurobasal-A Medium Thermo Fisher

Scientific Catalog #10888022

(NB-A)

supplemented with 10%

Fisher Catalog #10082147

After 5 days of culture, these cells are ready for further experiment

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