Jul 12, 2022

Primary cortical neuronal culture

Xiqun Chen[1], Qing Ye[2]

[1]Department of Neurology Massachusetts General Hospital Harvard Medical School Charlestown MA 02129 USA, Aligning Science Across Parkinson's (ASAP) Collaborative Research Network Chevy Chase MD 20815 USA; [2]Department of Neurology Massachusetts General Hospital Harvard Medical School Charlestown MA 02129 USA, Department of Neurology Longhua Hospital Shanghai University of Traditional Chinese Medicine Shanghai 200032 China

dx.doi.org/10.17504/protocols.io.b4t7qwrn

Pranay Srivastava

ABSTRACT

Primary cortical neurons were prepared from C57BL/6J mice embryonic day 17. The dissected cortical tissue was digested, triturated, and centrifuged. Cells were plated onto poly (L-lysine)-coated 24-well plates at 10^6 cells per well and cultured in NB-A with 2% B27 (Invitrogen, USA). After 24 h in culture, 5μg/ml cytarabine was added to inhibit the growth of glial cells in the medium and then changed to the original medium 48 h later. Neurons were cultured for 5 days and ready for experiments.

DOI

dx.doi.org/10.17504/protocols.io.b4t7qwrn

PROTOCOL CITATION

Xiqun Chen, Qing Ye 2022. Primary cortical neuronal culture. **protocols.io** https://dx.doi.org/10.17504/protocols.io.b4t7qwrn

KEYWORDS

ASAPCRN

CREATED

Feb 08, 2022

LAST MODIFIED

Jul 12, 2022

PROTOCOL INTEGER ID

57951

MATERIALS TEXT

DMEM, high glucose **Thermo Fisher**

DMEM/F12 **Scientific Catalog #11965092** Step 5

FBS

Fetal Bovine Serum, certified, heat inactivated, United States **Thermo Fisher Catalog #10082147** Step 5

Poly L Lysine

Neurobasal-A Medium **Thermo Fisher**

Neurobasal-A medium **Scientific Catalog #10888022**

B-27™ Supplement (50X), serum free **Gibco - Thermo**

B27 **Fisher Catalog #17504044** Step 8

Cytarabine

1 For Primary cortical neuronal 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**

4 Transfer the cortices to 15 ml tubes containing 1.5 ml trypsin–EDTA (0.25%) and incubate it at **37 °C** 15m 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** , **00:05:00**) and resuspend the pellet in 10ml 5m DMEM, high glucose **Thermo Fisher Scientific Catalog #11965092** medium supplemented with 10% Fetal Bovine Serum, certified, heat inactivated, United States **Thermo Fisher Catalog #10082147**

6 Triturate the cell suspension 10 times with a 1ml pipette

Citation: Xiqun Chen, Qing Ye Primary cortical neuronal culture https://dx.doi.org/10.17504/protocols.io.b4t7qwrn

7 Coat 24 well plate with Poly-L-Lysine **Contributed by users** (PLL) 1d

Place the cover glass at the bottom of the 24-well plate and add 400ul/well of PLL coating (0.01%) for 24h and incubate at **37 °C**

Wash 3 times with PBS after **24:00:00**

8 Seed the cells onto PLL coated 24-well plates at a cell density of 10^6 cells/well containing specialized Neurobasal™-A Medium **Thermo Fisher Catalog #10888022** supplemented with 2% B-27™ Supplement (50X), serum free **Gibco - Thermo Fisher Catalog #17504044** 1d

Incubate for **24:00:00** at **37 °C**

9 Post 24 h, add 2µg/well of cytarabine (Stock - 5µg/ml) to the culture to inhibit the glial cell growth.

10 **48:00:00** later remove the medium completely 2d

Add 400µl/well of NB-A supplemented with 2% B27

11 **Transduction with BRAF (Optional)**

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

12 After 5 days, the neurons were transduced with $BRAFV^{600E}$, or $BRAF^{WT}$, or vector lentivirus with 8 µg/ml polybrene (Sigma–Aldrich, USA) for 24 h.

13 The cells were cultured in NB-A for 120 h and used for subsequent experiments.

Citation: Xiqun Chen, Qing Ye Primary cortical neuronal culture https://dx.doi.org/10.17504/protocols.io.b4t7qwrn