Primary astrocyte culture

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ABSTRACT

Primary astrocytes were obtained from C57BL/6 mice embryonic day 17. The dissected cortical tissue was digested, triturated, and centrifuged. The cell pellet was resuspended in high-glucose DMEM/F12 supplemented with 10% FBS. Cells were seeded in poly-L-ornithine-coated Petri plates. Nonadherent cells were removed after 5-7 days.

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1 For primary astrocyte 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 15m **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** , **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

7 Count the cells and plate them in a density of 50,000 cells/cm^2 into PLL coated T-75 flask.

8 After 3 h, change the culture medium once and check the growth and survival of cells under the microscope.

9 Put the culture flask in a **37 °C** -cell incubator for **24:00:00** 1d

10 Change the media after **24:00:00** and then every **72:00:00** . A confluent layer of the astrocytes grows at the bottom in 5-7 days followed by a top layer of microglia. 4d

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11 After 5-7 days, put the flask on the shaker for 2 h at 200 rpm at 37°C (incubator). Collect and discard the supernatant (consisting of microglia and some oligodendrocytes).

12 Wash the adherent cells with PBS and add 0.25% trypsin at 37°C to detach the cells.
Immediately add **10 mL** DMEM medium containing 10% FBS

13 Centrifuge the cell suspension at **1500 rpm** min for **00:05:00** 5m

Resuspend the cells in a T75 flask or 24 well plate with high glucose DMEM supplemented with 10% FBS.

14 Identification of astrocytes by immunofluorescence staining: Use GFAP antibody (astrocytic marker) to identify the population of astroctes

15 **Transduction with BRAF** (Optional)
Astrocytes were transduced with $BRAFV^{600E}$, or $BRAF^{WT}$, or vector lentivirus plus 8 μg/ml polybrene for 24 h.

16 After transduction, the cells were cultured for 120 h in high-glucose DMEM/F12 (400ul/well) with or without FBS, and the medium as well as the cells were collected for subsequent experiments.

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