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Primary microglial culture

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ABSTRACT

Mixed glial cells were obtained from C57BL/6 mice embryonic day 17. Cells were cultured in high-glucose DMEM/F12 supplemented with 10% FBS in humidified air containing 5% CO2 at 37°C. The culture medium was replaced with fresh medium 24 h after the initial preparation and every 3 days thereafter. After 1 week, microglial cells were obtained by mechanical shaking of the mixed glial cell cultures for 1 h. Cells were routinely monitored for purity by ionized calcium-binding adaptor molecule 1 (Iba1) staining and the population of Iba1+ cells was >95%

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1 Primary microglial 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 (**400 x g** , **00:05:00**). Aspirate the media and resuspend the pellet in 5 ml 5m
DMEM, high glucose **Thermo Fisher Scientific Catalog #11965092**

6 Count the cells and plate them in a density of 50,000 cells/cm^2 into PLL coated T-75 flask.
Makeup the volume to 15 ml with the
DMEM, high glucose **Thermo Fisher Scientific Catalog #11965092**

7 Change the culture medium the next day followed by the addition of a fresh culture medium 1w
every **168:00:00** till 14 days of culture

8 Put the flask on a shaker for **06:00:00** at **37 °C** (The microglia grow as a monolayer on the top) 8h 5m
Collect the detached cells, centrifuge (**400 x g, Room temperature** , **00:05:00**) and

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resuspended in **4 mL** of

DMEM, high glucose **Thermo Fisher Scientific Catalog #11965092**

Count the cells and plate at a density of 50,000 cells/cm^2 in a 24 well plate
Incubate them at **37 °C** in 5% CO2. After **02:00:00** microglia usually attaches to the bottom of the plate.

The purity of the cells was monitored by staining with ionized calcium-binding adaptor molecule 1 (Iba1) antibody
(Microglia obtained by this method have a purity of 90%-95% and can be identified by immunofluorescence staining.

9 **Transduction with BRAF (Optional)** 1d

The purified microglial cells were seeded at a density of 80% and transduced with $BRAFV^{600E}$, or $BRAF^{WT}$, or vector lentivirus with 8 μg/ml polybrene (Sigma–Aldrich, USA) for **24:00:00** .

10 After transduction, the cells were cultured in DMEM, high glucose **Thermo Fisher Scientific Catalog #11965092** with or Fetal Bovine Serum without (FBS) **ATCC Catalog #30-2020** for **120:00:00** , and the medium, as well as the cells, were collected for subsequent experiments. 5d

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