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# Primary Astrocyte Culture

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**We use this protocol and it's working**

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## Abstract

This protocol is for the isolation and preparation of primary cortical astrocytes cultures from the pups of C57BL/6 mice.

## Primary Astrocyte Culture

1 Day 1: Take 6 newborn mice (1-3 days postpartum) back to lab

2 Dissect out the brain and place in a 50 mL conical that has 10 mL of cold washing media, and keep on ice

3 Repeat steps 2 until all 6 brains are out and pool into the same conical

4 Take a new 50 mL conical and place a 40 um strainer inside it

5 Dump all 10 mL of cold washing media plus the brains into the strainer and smash the brains through the strainer with the plunger of a 5 mL syringe

6 Wash the filter with 10 mL of cold washing media

7 Spin samples at 2,000 rpm for 10 minutes at 4°C

8 Decant media and wash with 20 mL of cold washing media

9 Spin samples at 2,000 rpm for 10 minutes at 4°C

10 Repeat steps 9 and 10

11 Decant media and resuspend samples in 36 mL of pre-warmed complete astrocyte media

12 Plate 6 mL of cell mixture to a T-25 flask keeping the cap loose

13 Day 3: Dump out media and add 8 mL of pre-warmed completed astrocyte media

14 Day 5: Dump out and add 8 mL of pre-warmed completed astrocyte media

15 Day 7: Dump out and add 8 mL of pre-warmed completed astrocyte media

16 Day 8: Shake flasks for 1.5 to 2 hours at 260 rpm at 37°C

17 Take off the media and add 6 mL of pre-warmed complete astrocyte media

18 Shake flasks at 100 rpm for 24 hours at 37°C

19 Day 9: Aspirate the media with dislodged cells and wash with 5 mL of pre-warmed HBSS

20 Aspirate the HBSS

21 Add 1-2 mL of pre-warmed Trypsin EDTA and leave in the 37°C incubator until cells are lifted

22 Add 5 mL of pre-warmed complete astrocyte media to stop the reaction

23 Pipette all the flasks in to one 50mL conical tube and centrifuge at 1,200 rpm for 5 minutes at 20°C

24 Dump out the supernatant and resuspend the pellet in 20 mL of pre-warmed complete astrocyte media

25 Count cells using automatic cell counter and resuspend the cells to 1×10^6 - 3×10^6 cells/mL for plating

26 Plate 1×10^6 cells/mL per T-75 vented flask with 20 mL of pre-warmed media

27 When cells are 100% confluent, parafilm the vented caps and can store cells in mammalian incubator for up to 2 months