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Astrocyte isolation and culturing

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ABSTRACT

This protocol details steps for preparing an astrocyte monoculture from mouse pups. From dissection to plated samples ready to be transfected, treated, lysed, or imaged, the protocol takes 20-25 days.

BEFORE START INSTRUCTIONS

The night before (could also do morning of, but makes your life easier):

1. Locate tools and put next to the microscope.
2. Aliquot Dissection Media (DM). Need two tubes- one 50mL-tube containing media needed to fill plates for the actual dissection (~ 30 mL) and another 50mL tube containing either 4.5 mL - 9 mL media (depending on how many pups are being dissected- 4.5 is sufficient for less than 6 pups) to put dissected cortices into for trypsin.

Breakdown of how the dissection media is used:

- Tube 1 (~ 30 mL):
  - 10 mL for each of two 10cm dishes to do the dissection in (20 mL total)
  - 4 mL for each of two dishes to rinse pup heads after an EtOH wash, before dissection (8 mL total)
- Tube 2, for dissected corticies:
  - For less than 6 pups, 4.5 mL (and add 500 µL [M] 2.5 % (v/v) trypsin for digestion)
  - For more than 6 pups, 9 mL (and add 1 mL [M] 2.5 % (v/v) trypsin for digestion)

1. Aliquot glial media:

- 4 mL per flask.
- 15 mL for the three washes following trypsin digestion.
- 5 mL to neutralize trypsin for spin.

Protocol status: Working
We use this protocol and it's working

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1. Poly-D-Lysine coat flasks (1 flask/pup you plan to dissect) and store at 4 °C Overnight:
   - Mix Poly-D-Lysine (ThermoFisher,) with PBS -Ca^{2+}, -Mg^{2+}
   - You can also do this the morning of the dissection, but they must sit for 01:00:00 at room temperature to allow for sufficient coating.

The morning of the dissection:

1. Submerge tools in EtOH.
2. Make 4 dissection media filled plates and 1 EtOH plate:
   - One 5cm dish ([M] 100 % (v/v) Sterile EtOH)
   - Two 5cm dishes (4 mL DM each)
   - Two 10cm dishes (10 mL DM each)
3. Place 50mL tubes containing DM on ice.

Dissection

1 Sacrifice mouse pups postnatal day (P) 1-3 via isoflourane overdose.

2 Decapitate pups and transfer heads to a dish with [M] 100 % (v/v) Sterile EtOH. Swirl to clean.

3 Transfer heads to dish of dissection media and swirl again. Do this one more time.

4 Transfer heads to a new 10cm dish and place whole dish in a cell culture dish with a wider diameter. The surrounding dish should then be filled with ice to keep the heads chilled while dissecting.

5 Using the dissection microscope to magnify the procedure, remove skin from heads by tearing with forceps and pushing it down and to the side

6 Using *spring-loaded scissors,* make a sagittal cut through the skull, down the median plane, starting from the base near where the spinal cord would be. At the end of the cut, twist the scissors slightly so they're diagonal behind the eye sockets and make one more, very short cut on either side. This helps you open up the skull.

7 Make two coronal cuts in the middle of the skull where the skull plates meet on either side of your original sagittal cut.

8 Using forceps, Gently open up the skull flaps you just created. If you're taking cortex with you, you can gently slide the forceps under the skull and move it back and forth to disconnect them.

9 Once the ventral half of the skull is open, push off the dorsal part a little bit.

10 Dig your forceps diagonally under the brain and into the underneath part of the skull, toward each other. Once they're under, firmly pull apart and the brain will come free.

11 Move the brains to a fresh dish on ice once they're free of the skulls.

12 Bisect the brains so you have the two hemispheres. Unroll them to open them up one at a time, with the inside of the hemisphere initially facing up. Can make a cut with the forceps where the crease is that makes them re-roll to prevent this.

13 Remove all of the meninges from the inside. Also remove the hippocampus.

14 Flip hemisphere. Remove the rest of the meninges. Remove cortex area of the hemisphere, being careful to leave midbrain components.

15 10. Put cortices into the tube of dissection media.

Plating brain cells in flasks

16 Using a pasteur pipette and bulb, triturate cortices in the dissection media a few times to make the chunks of cortex a little smaller.

17 Add 0.5 mL [M] 2.5 % (v/v) trypsin per 4.5 mL dissection media and incubate for 00:07:00 . 7m

18 Add 5 mL glial maintenance media to neutralize trypsin and centrifuge for 00:04:00 at 1.1gxs. 4m

19 While cells are spinning, remove PDL solution from pre-prepared flasks (see Before Start section

of protocol) and rinse 2x with sterile ddH2O. Put 3 mL media in each flask.

20 GENTLY suck off the trypsin with a serological pipette and replace with 5 mL DMEM to wash cortices. Wait for the cortices to sink back to the bottom.

21 Repeat DMEM wash two more times for a total of three washes.

22 After the final wash, add 2 mL glial media.

23 Homogenize with a Pasteur pipette attached to a bulb, and then using a P1000.

24 Put cell suspension through 100 µm cell strainer.

> Note
>
> any cell strainer with mesh size greater than 100 µm is sufficient

25 Supplement to desired volume with more media and mix by gently inverting.

26 10. Plate cells on T25 flasks.

Glia maintenance

1w 6d

27 ~6-10 hours after dissecting, firmly whack the side of each flask with your palm one time to loosen cell debris. Immediately following this, replace media.

28 Change media every 72:00:00 . 3d

Note

Could be more or less depending on what cells look like they need, but the cell strainer and same-day whack and media change helps eliminate initial debris.

Note

Cells should look good around 10-14 days.

Purifying astrocytes

29 Depending on the type of T25, either tighten cap so it's airtight OR parafilm over the top of the cap and around the sides to ensure no air exchange and maintain 5% CO2 within the flask.

30 Tape flask to a shaker, ensuring media is covering cells.

31 Shake for 04:00:00 - 06:00:00 at 37 °C, 250 rpm. 6h

Plating cultured glia

1h 12m

32 Prepare new PDL surfaces for glia at least 01:00:00 before starting. 1h

> Note
>
> can also do this step the night before and put plates in refrigerator

33 Warm 2.5 mL [M] 0.05 % (v/v) Trypsin-EDTA and glial maintenance media in T25 flasks.

> Note
>
> Make sure it's super warm before you use it! This helps the cells come off easier.

34 Remove flasks from shaker and hit them firmly with your palm ~3-4 times to help dislodge stubborn contaminating cell types.

35 Aspirate media from flasks.

36 Wash 2x with PBS w/o magnesium and calcium and aspirate.

37 Add 1.5mL trypsin-EDTA to each flask and put in the incubator for 00:07:00 - 00:08:00 8m
.

38 Remove flask- when cells are ready to come off, they should look shriveled but still attached under a microscope.

39 Smack the flask firmly with your palm 4-6 times to knock cells loose.

40 Use a 5mL strippette to get in the flask and rinse the rest of the cells off

41 Remove cell suspension and put in a 50mL conical.

42 Add glial maintenance media ~1:1 to the trypsin-EDTA cell suspension in the 50mL conical to neutralize trypsin.

43 Spin at 1.1 gxs for 00:04:00 . 4m

44 Aspirate trypsin and resuspend cells in glial media using a p1000.

Note

To do this, add 1 mL of media and resuspend with a p1000 until homogenous, then add more glial media (~ 4 mL more) to bring cell concentration into a range where cell count accuracy is attainable by the countess cell counter. Invert to mix and count cells. Remember to record % viability.

45 Plate astrocytes at 175,000 LIVE cells per imaging plate.