



May 10, 2023

Isolation of sex specific PND 1/2 rat cortical primary astrocytes

DOI

dx.doi.org/10.17504/protocols.io.e6nvwjry7lmk/v1

Neha Paranjape¹, Jonathan A. Doorn¹, Hans-Joachim Lehmler¹, Laura E Dean¹

¹University of Iowa



Hans-Joachim Lehmler

University of Iowa

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.e6nvwjry7lmk/v1>

Protocol Citation: Neha Paranjape, Jonathan A. Doorn, Hans-Joachim Lehmler, Laura E Dean 2023. Isolation of sex specific PND 1/2 rat cortical primary astrocytes. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.e6nvwjry7lmk/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: April 27, 2023

Last Modified: May 10, 2023

Protocol Integer ID: 81114

Keywords: Astrocytes, purified culture of primary astrocyte, primary astrocyte, developing astrocyte, confluent with only astrocyte, including astrocyte, only astrocyte, primary glial cell, flow cytometry, microglia, cytometry, abundant cell type in the brain, gfap primary antibody, cells per flask, many xenobiotic metabolizing enzyme, cell, primary antibody, cortical primary

Funders Acknowledgements:

Iowa Superfund Research Program

Grant ID: P42 ES013661

Abstract

Many xenobiotic metabolizing enzymes, such as P450s, SULTs, UGTs, and sulfatases, are expressed in the brain, including astrocytes, the most abundant cell type in the brain. To obtain a purified culture of primary astrocytes, a method was developed and confirmed through flow cytometry. Primary glial cells are isolated separately from male and female postnatal day 1/2 (PND1/2) rat pups to isolate developing astrocytes. Sex is first determined by the anogenital distance and then confirmed through PCR followed by gel electrophoresis. Once isolated, the medium is changed every 3-4 days with additional incubation at room temperature for 10 min on a plate shaker before aspirating media to release microglia. When flasks reach confluency, cells are passaged and reseeded with 2×10^5 cells per flask. Once the cells reached passage 3, or appear to be confluent with only astrocytes, flow cytometry is performed utilizing Anti-Iba1 and GFAP primary antibodies.

Materials

Chemical List:

S-MEM (minimal essential medium with Earle's salts, modified for suspension cultures; ThermoFisher, 11380-037)
Pen/strep (Live Technologies; 100 U/mL penicillin, 100 µg/L streptomycin final; ThermoFisher, 10378-016)
3 U/mL dispase (ThermoFisher, 17105-041)
MEM (minimal essential medium with Earle's salts; ThermoFisher, 11095080)
Heat-inactivated horse serum (ThermoFisher, 26050-088)
15000 U DNase I from bovine pancreas (type IV; Sigma 69182)
Sigmacote (Sigma, SL2-100ML)
Trypan blue (ThermoFisher, 15250-061)
Extract-N-Amp Tissue PCR kit (Millipore Sigma, XNAT2)
DNase- and RNase-free water (ThermoFisher, 10977-015)
Agarose (ThermoFisher, 18300-012)
10x TAE buffer(ThermoFisher, 15558-042)
EtBr (ThermoFisher, 15585-011)
DNA ladder for gel electrophoresis (ThermoFisher, 10416-014)
Gel electrophoresis loading buffer (ThermoFisher, 10816015)
Anti-Iba1 antibody (EPR16588) (ab178846) for flow cytometry (Abcam, (EPR16588) (ab178846))
Anti-GFAP (Cell Signaling 3670) (Cell Signaling Technology, 3670T)
Donkey Serum (Millipore Sigma, D9663-10ML)
Goat serum (ThermoFisher, 50197Z)
DPBS (ThermoFisher, 14190144)
1x Trypsin EDTA (0.25%) (ThermoFisher, 25200056)
Paraformaldehyde (diluted to 2%) (ThermoFisher, J19943.K2)
0.05% Triton-X in PBS (ThermoFisher, HFH10)
Goat anti-rabbit 488 (Life Tech) (diluted 1:500) (ThermoFisher, A-11008)
Donkey anti-mouse 647 (Life Tech) (diluted 1:200) (ThermoFisher, A-31571)
Autoclaved water

Accessories:

Filter flask (150 mL, 0.2 µm pore size, cellulose acetate) (Corning, 431154)
Filter flask (1 L, 0.2 µm pore size, cellulose acetate) (Corning, 431098)
0.2 µm syringe filter (cellulose acetate) (Millipore Sigma, WHA10462200)
Pasteur pipets (Fisher Scientific, 22-183632)
Cotton-tipped swabs (1 per Pasteur pipet)(Fisher Scientific, 22-363-157)
Fine-angled micro-dissecting scissors (Fisher Scientific, 08-953-1B)
Forceps for dissection(Fisher Scientific, 08-953F)
Scissors for dissection (Fisher Scientific, 13-804-18)
Sterile gauze pad (1 per excised brain) (Fisher Scientific, 19-090-729)
50 mL conical polypropylene tube (1 per brain section or 1 per 10 brains from PND1/2) (Fisher Scientific, 14-432-22)
15 mL conical polystyrene centrifuge tubes (5 per 50 mL beaker) (Fisher Scientific, 14-959-53A)

10 mL glass serological pipet (Fisher Scientific, NC9868325)
T75 cell culture flasks (Fisher Scientific 50-809-260)
1.5 mL centrifuge tube (1 per pup) (Fisher Scientific, 05-408-129)
12×75 mm falcon tubes (Corning, 352052)
50 mL beaker with stir bar autoclaved (1 per 50 mL conical polypropylene tube)
Ice bucket

Instruments:

Stir plate (FisherSci 11520495H)
37°C, 95% air/5% CO₂, 95% relative humidity cell culture incubator (Hera cell 150, 51026334)
Inverted phase-contrast microscope (Olympus CKX41)
Eppendorf centrifuge 5810 R (5811 08785)
Swinging bucket rotor (Eppendorf A-4-62 Swing Bucket Rotor; Fisher Scientific 05-414-138)
Hot water bath set to 37°C (Fisher Scientific, Isotemp Model 2320)
Autoclave (Easter Services, Inc. 1620E)
Portable power pipettor (Eppendorf easypet 4430000018)
Gel electrophoresis power supply, instrument, and combs (ThermoFisher PS0300; ThermoFisher A2-BP)
Hot water bath set to 95°C (Fisher Scientific, Isotemp 210, 15-462-10)
Biosafety cabinet (The Baker Company, SG403)
BD Accuri™ C6 Flow Cytometer (BD Biosciences, 660519)
BD Accuri C6 Software
Bunsen burner
Microwave

Troubleshooting



Before you begin

- 1 Prepare **complete S-MEM**
 - 1.1 Add 5 mL pen/strep solution to the 500 mL S-MEM.
 - 1.2 Store complete S-MEM medium for up to 2 months at 4°C
- 2 Prepare **dissociation medium** and warm to 37°C - MUST MAKE FRESH EACH ISOLATION DAY. Prepare 10 mL of dissociation medium per sex per litter of PND1/2 pups (20 mL total).
 - 2.1 Add **187.5 U dispase for cortex isolation** per 125 mL complete S-MEM in an autoclaved container.
 - 2.2 Sterilize with a 150 mL sterile filter flask (0.2 µm pore size, cellulose acetate)
 - 2.3 Prewarm in 37°C hot water bath for 1 hour before use.
- 3 Prepare **astrocyte growth medium**.
 - 3.1 Add 900 mL MEM, 10 mL pen/strep, and 100 mL heat-inactivated horse serum to a 1 L sterile filter flask.
 - 3.2 Sterilize with a 1 L sterile filter flask (0.2 µm pore size, cellulose acetate) by pulling vacuum through the apparatus.
 - 3.3 Store the astrocyte growth medium for up to 1 month at 4°C.
- 4 Prepare **DNase I solution, 8000 U/mL**.
 - 4.1 Add 15,000 U DNase I from bovine pancreas (type IV) to 1.875 mL autoclaved water.

- 4.2 Mix gently, do NOT vortex. DNase I from pancreas is vulnerable to inactivation by physical damage and should not be vortexed.
- 4.3 Sterilize with a 0.2 μ m syringe filter (cellulose acetate).
- 4.4 Store in 100 μ L aliquots at -20°C.
- 5 Fire polish and Sigmacote treatment of pipets for cell isolation.
 - 5.1 **Fire polish the tip of a 9-in Pasteur pipet by placing the small end in an open flame from a Bunsen burner for a few seconds.** This will slightly melt the glass and will produce a smooth tip to the pipet, which helps prevent damage to the cells. However, if the opening becomes too small, increased shear forces during titration may lead to increased cell damage.
 - 5.2 Plug the large end of the fire-polished Pasteur pipets with cotton removed from a cotton-tipped swab or, alternatively, small pieces of cotton balls.
 - 5.3 Treat fire-polished Pasteur pipets and 10 mL glass, cotton-plugged serological pipets with Sigmacote by drawing the viscous Sigmacote solution up into the pipet with a portable power pipettor. Do not let the solution touch the cotton plug. Allow Sigmacote to drain back into the bottle, and allow the pipets to drain and dry completely in a beaker lined with paper towels.
 - 5.4 Place Sigmacoted pipets in a metal container with lid and autoclave.
- 6 Autoclave fine-angled micro-dissecting scissors, large scissors, and forceps in a beaker wrapped with aluminum foil.

Brain and Tail Dissection

- 7 Sex the pups based on anogenital distance (females will have a much shorter distance than males, compare pups to one another to be able to sex pups efficiently).
- 8 For pups at PND1/2, use between 8 and 15 pups and **decapitate using sterile large scissors.**



- 9 Place the head on a sterile gauze pad. Each head will be placed on a separate gauze pad.
- 10 Take a tail snip of approximately 0.5-1 cm (too much tissue may not be digested with the DNA extraction kit in the steps to follow) and place the tail in a 1.5 mL centrifuge tube, label the tube according to the sex of the pup, and then place it on ice.
- 11 Remove brain from head.
 - 11.1 Secure the head by holding down the snout.
 - 11.2 With fine-angled micro-dissecting scissors, cut the skin along the midline from the base of the skull to the eyes.
 - 11.3 Use 0.8 mm forceps to separate skin and expose the skull as necessary.
 - 11.4 Cut skull along midline with micro-dissecting scissors.
 - 11.5 Cut away the skull flaps for easier removal of the brain, remove the brain with spatula, and place the brain on a sterile gauze pad.

Brain Tissue Dissection for Cortex

- 12 Split the brain in half down the midline to separate each hemisphere from one another. Place each hemisphere with the inside facing up to examine the hippocampus on the sterile gauze pad.
- 13 Gently remove darker hippocampal crescents with forceps (dissecting microscope can be used here to assist in locating the different brain regions).
- 14 Remove remaining meninges with forceps (look for any veins and flip the brain section over a few times to use the sterile gauze pad to help remove the meninges).
- 15 Place sections in a sterile 50 mL conical polypropylene tube containing 10 mL complete S-MEM on ice labeled with the proper sex



- 16 Repeat steps, placing all cortical sections in a single 50 mL tube on ice per sex (all pups from the same litter with the same sex can be placed in the same 50 mL tube).

Confirm sex of pups

- 17 Add 100 μ L extraction solution from Extract-N-Amp Tissue PCR kit to each centrifuge tube containing a small tail snip for a pup (collected in step 10).
- 18 Add 25 μ L tissue preparation solution from Extract-N-Amp Tissue PCR kit
- 19 Incubate at room temperature for 10 min
- 20 Incubate in 95°C hot water bath for 3 min
- 21 Remove from hot water bath and add 100 μ L neutralization solution B from Extract-N-Amp Tissue PCR kit
- 22 Vortex
- 23 Remove tail snip and discard
- 24 Store tissue extract at 4°C or use for PCR immediately
- 25 PCR amplification of *Sry* gene
 - 25.1 Prepare master mix with the following per tissue extract: **10 μ L** extract-N-amp PCR reaction mix, **4 μ L** RNase- and DNase-free water, **1 μ L** of 25 μ M forward primer (5'- TAC AGC CTG AGG ACA TAT TA -3'), **1 μ L** of 25 μ M reverse primer (5'- GCA CTT TAA CCC TTC GAT GA -3'). An autosomal gene, *Actb* that encodes for beta-actin was used as control with the following set of primers: Forward: 5'- AGC CAT GTA CGT AGC CAT CC -3'; Reverse: 5'- TGT GGT GGT GAA GCT GTA GC -3'.
 - 25.2 To PCR tubes labeled for each tail snip extract, add 16 μ L of master mix



- 25.3 Add 4 μ L tissue extract to each designated PCR tube containing the master mix
- 26 Run PCR program
 - 26.1 94°C for 5 min
 - 26.2 35 cycles of: 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 1 min
 - 26.3 72°C for 7 min
- 27 Prepare 1% agarose gel
 - 27.1 Prepare 1x TAE buffer by diluting 10x TAE buffer with DI water (1:10).
 - 27.2 Add 1 g agarose to 100 mL 1x TAE buffer
 - 27.3 Microwave in 1 min intervals, swirling the flask each min until clear
 - 27.4 Let agarose cool to ~50°C (warm to touch and able to grab)
 - 27.5 Add 2 μ L EtBR or use another nucleic acid stain, such as GelRed per manufacturer's instructions such that final stain concentration is 1x.
 - 27.6 Pour gel into gel tray with well comb(s) in place
 - 27.7 Let cool at room temperature for 1 hour (until solidified)



- 27.8 When solidified, remove the combs carefully without damaging the gel.
- 27.9 Orient the gel in the gel electrophoresis apparatus such that the combs are placed towards the cathode. The DNA will be run from cathode to anode.
- 28 Fill the gel electrophoresis box with 1xTAE buffer until the gel is fully covered
- 29 To each PCR-amplified DNA sample (20 μ L), add 4 μ L of loading dye (6x concentration).
- 30 Carefully load 10 μ L of the ladder followed by samples to each well
- 31 Run gel at 100 V for 1 hour
- 32 Turn the power off and disconnect the electrodes
- 33 Visualize under UV light to confirm sex of pups. The samples for males should show a band for *Sry* gene, amplified product of 317 bp while all samples should show a band for *Actb* gene, amplified product of 220 bp.

Dissociate cells extraction

- 34 Carefully remove as much complete S-MEM as possible with a sterile, cotton-plugged, 9' Pasteur pipet, taking care to retain all pooled brain sections.
- 35 Add 7 mL **prewarmed dissociation medium** to a 50 mL sterile beaker with a stir bar and carefully pour sections into the beaker.
- 36 Gently triturate the sections 7-8 times using a Sigmacote-treated 10 mL serological pipet.
- 37 Stir for 10 min at low speed on a stir plate inside the biosafety cabinet.



- 38 While this is stirring, prepare 5 of the 15 mL centrifuge tubes for each extraction by adding 5 mL room temperature astrocyte growth medium to each tube.
- 39 Additionally, thaw DNase I solution and place on ice
- 40 After 10 min of gentle stirring, remove the beaker from the stir plate and place at ~45° angle for 3 min to allow the non-dissociated tissue to collect at the bottom of the beaker (e.g. using a 1.5 mL centrifuge tube holder).
- 41 Carefully aspirate the dissociated cells in medium with a Sigmacote-treated 10 mL glass serological pipet (take care not to remove the undissociated tissue pieces).
- 42 Place this suspension into a 15 mL centrifuge tube that contains astrocyte growth medium
- 43 Invert the 15 mL centrifuge tube 2-3 times to mix and then allow the tube to sit undisturbed during the continuing extractions.
- 44 Add 5 mL prewarmed dissociation medium to the 50 mL beaker containing undissociated tissue pieces.
- 45 Add 100 μ L of 8000 U/mL DNase I solution
- 46 Stir for 10 min at low speed on a stir plate inside the biosafety cabinet.
- 47 After 10 min of gentle stirring, remove the beaker from the stir plate and place at ~45° angle for 3 min to allow the non-dissociated tissue to collect at the bottom of the beaker (using a 1.5 mL centrifuge tube holder works nicely here).
- 48 Carefully aspirate the dissociated cells in medium with a Sigmacote-treated 10 mL glass serological pipette (take care not to remove the undissociated tissue pieces).
- 49 Place this suspension into a 15 mL centrifuge tube that contains astrocyte growth medium.
- 50 Invert the 15 mL centrifuge tube 2-3 times to mix and then allow the tube to sit undisturbed during the continuing extractions.



- 51 Add 5 mL prewarmed dissociation medium to the 50 mL beaker containing undissociated tissue pieces. Do not add any more DNase I solution for the remaining extractions.
- 52 Once you have dissociated a total of 3 times, remove undissociated tissue from the 15 mL tubes by inserting a sterile, fire-polished, cotton-plugged, Sigmacote-treated 9" Pasteur pipette to the bottom of the tube and carefully aspirate the undissociated tissue.
- 53 Place this tissue back into the 50 mL beaker and perform the final two extractions, adding another 5 mL prewarmed dissociation medium to the 50 mL beaker each time.

Pellet Cells

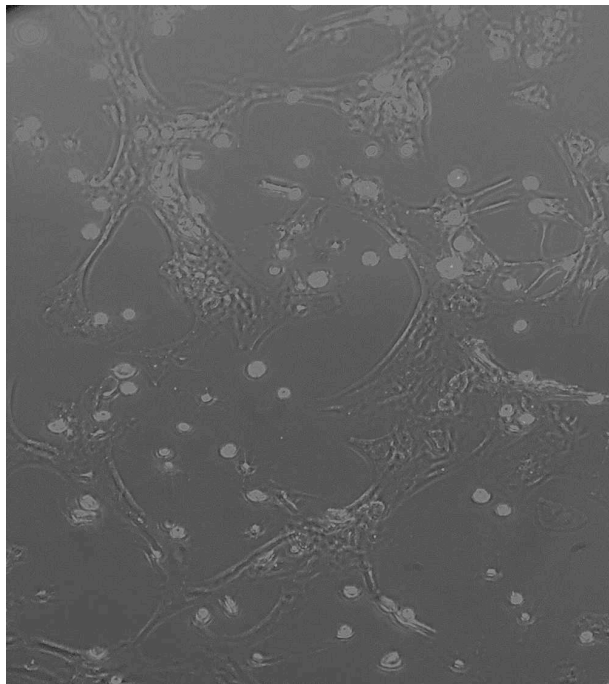
- 54 Pool dissociated cells and medium from all 15 mL centrifuge tubes into a singular 50 mL conical tube.
- 55 Centrifuge for 10 min at 1000 rcf at 4°C, in a swinging bucket rotor to pellet the suspended cells.
- 56 Carefully aspirate medium from the cell pellet.
- 57 Resuspend in 20 mL **astrocyte growth medium** by gently pipetting with a Sigmacote-treated 10 mL glass serological pipette.
- 58 Allow cells to sit for 5 min and then discard any sedimented tissue from the bottom of the tube by using a fire-polished Sigmacoted 9" Pasteur pipette.

Assess cell number and viability

- 59 Gently mix 100 µL cell suspension with 100 µL of 0.08% trypan blue staining solution
- 60 Allow cells to take up the trypan blue for 3 min
- 61 Determine the total cell number and cell viability with a hemacytometer and an inverted phase-contrast microscope (intact cells are able to exclude trypan blue while dead or damaged cells retain the dye).

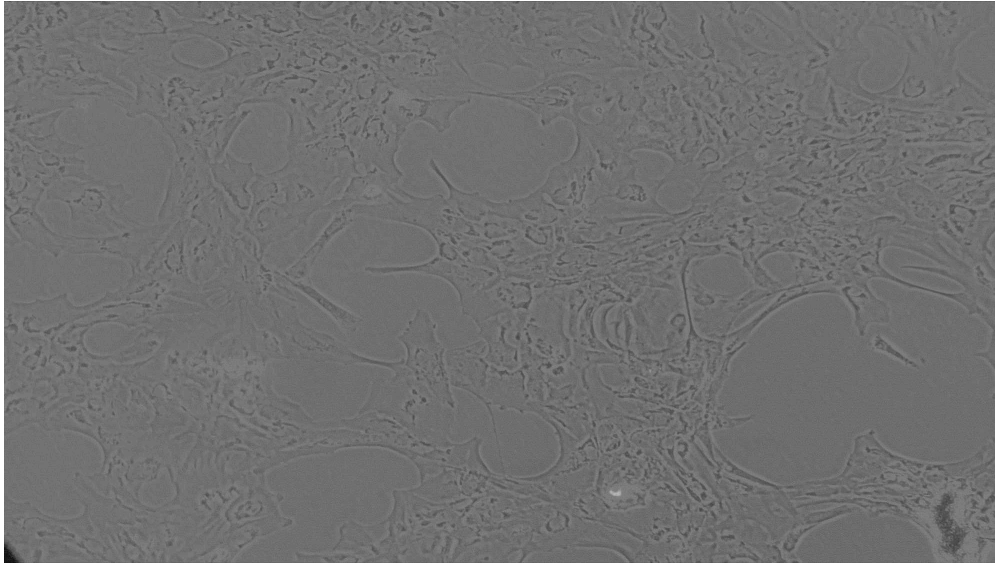
Plate and grow cells

- 62 Plate 5+ T75 cell culture flasks with 7.5×10^6 cells per flask in 9 mL astrocyte growth medium.
- 63 Place plated cells in a 37°C, 95% air/5% CO₂, 95% relative humidity incubator.
- 64 After 18-24 hours, remove the old growth medium with a sterile 5" Pasteur pipette attached to a vacuum source.
- 65 Add 9 mL of fresh astrocyte growth medium to each flask.
- 66 Change medium twice per week (every 3-4 days) as described below.
 - 66.1 For primary astrocytes that are passage 0-2 (P0-P2), check cells under the microscope, shake flasks for 10 minutes at a medium-low speed on a plate shaker, check cells under the microscope again to make sure astrocytes are still adhering to the flask while the microglia have popped off, aspirate media, add 5 mL DPBS per T75 flask, rinse plate well by moving DPBS back and forth across flask bottom, aspirate, add 9 mL growth media per T75 flask, and then place back in the 37°C, 95% air/5% CO₂, 95% relative humidity incubator.



Glial cells. All round cells are microglia that will "pop" off the flask with shaking.

- 66.2 For primary astrocytes that are either confluent astrocytes, or are passage 3+ (P3+), check cells under the microscope, aspirate, add 9 mL growth media per T75 flask, check cells once again under the microscope, and then place back in the 37°C, 95% air/5% CO₂, 95% relative humidity incubator.



Pure astrocyte culture.

Flow Cytometry to confirm purity of astrocytes

- 67 Harvest and wash cells for flow cytometry
- 67.1 Aspirate media from T75 flask
- 67.2 Rinse plate with 10 mL DPBS
- 67.3 Aspirate DPBS
- 67.4 Add 2 mL 37°C 1x trypsin EDTA (0.25%)
- 67.5 Incubate for 5 min in a 37°C, 95% air/5% CO₂, 95% relative humidity incubator



- 67.6 Tap sides of flask to allow cells to detach
- 67.7 Add 10 mL **FACS media** (DPBS + 2% heat-inactivated horse serum)
- 67.8 Centrifuge at 4000 rcf at 4°C, in a swinging bucket rotor for 10 minutes to pellet cells
- 67.9 Aspirate media
- 67.10 Resuspend in approximately 1 mL of fresh FACS media
- 67.11 Adjust cell number to a concentration of $1-5 \times 10^6$ cells/mL
- 67.12 Transfer 200 uL cell suspension to polystyrene round-bottom 12×75 mm falcon tubes
- 67.13 Centrifuge at 4000 rcf at 4°C, in a swinging bucket rotor for 10 minutes to pellet cells
- 67.14 Add 200 uL/well paraformaldehyde (2%)
- 67.15 Incubate at room temperature for 15 min on shaker
- 67.16 Centrifuge at 4000 rcf for 5 min at 4°C
- 67.17 Aspirate media using a fire-polished, cotton-plugged Pasteur pipette
- 67.18 Permeabilize (remove more cellular membrane lipids to allow large molecules like antibodies to get inside the cell easily) with 200 uL 0.05% Triton-X (in PBS)

- 67.19 Incubate at room temperature for 5 min on shaker
- 67.20 Centrifuge at 4000 rcf at 4°C, in a swinging bucket rotor for 5 minutes to pellet cells
- 67.21 Aspirate media using a fire-polished, cotton-plugged Pasteur pipette
- 68 Blocking the non-specific binding of the staining antibody reagents
 - 68.1 Prepare block solution: 1% donkey serum, 1% goat serum, 1x PBS; 490 µL PBS + 5 µL donkey serum + 5 µL goat serum
 - 68.2 Add 100 µL blocking solution to each washed tube
 - 68.3 Block for 20 min on ice
 - 68.4 Centrifuge at 4000 rcf at 4°C, in a swinging bucket rotor for 5 minutes to pellet cells
 - 68.5 Aspirate blocking solution using fire-polished, cotton-plugged Pasteur pipet
- 69 Addition of primary antibody
 - 69.1 Add 0.1-10 µg/mL (100 uL) of each primary labeled antibody (we use primary antibody diluted 1:200 in blocking solution).
 - 69.2 Incubate primary antibody for at least 30 min at room temperature
 - 69.3 Centrifuge at 4000 rcf at 4°C, in a swinging bucket rotor for 5 minutes to pellet cells
 - 69.4 Aspirate primary antibody solution using fire-polished, cotton-plugged Pasteur pipette



70 Addition of secondary antibody

70.1 Dilute secondary antibodies in blocking solution according to dilution according to dilution factor listed in materials.

70.2 Resuspend cells in secondary antibodies (100 uL each)

70.3 Incubate for 1 hour at room temperature in the dark (cover with aluminum foil and turn lights off)

71 Wash 3x

71.1 Centrifuge at 4000 rcf at 4°C, in a swinging bucket rotor for 5 minutes to pellet cells

71.2 Aspirate antibodies using fire polished, cotton-plugged Pasteur pipette

71.3 Resuspend in 200 µL of ice-cold FACS media

72 Analyze on flow cytometer as soon as possible. Flow cytometry data analysis will involve identifying a population of cells through subsequent steps called gating and subjecting that population to various parameters through subsequent gating steps.

72.1 First, make forward scatter (FSC-A) and side scatter (SSC-A) density plots for the cell events using the BD Accuri C6 Software. The first gate will be applied to remove debris (dense accumulation of events on the bottom left corner). Thus, the gate will select all events measured by the flow cytometer except the debris, these events should all therefore be cells.

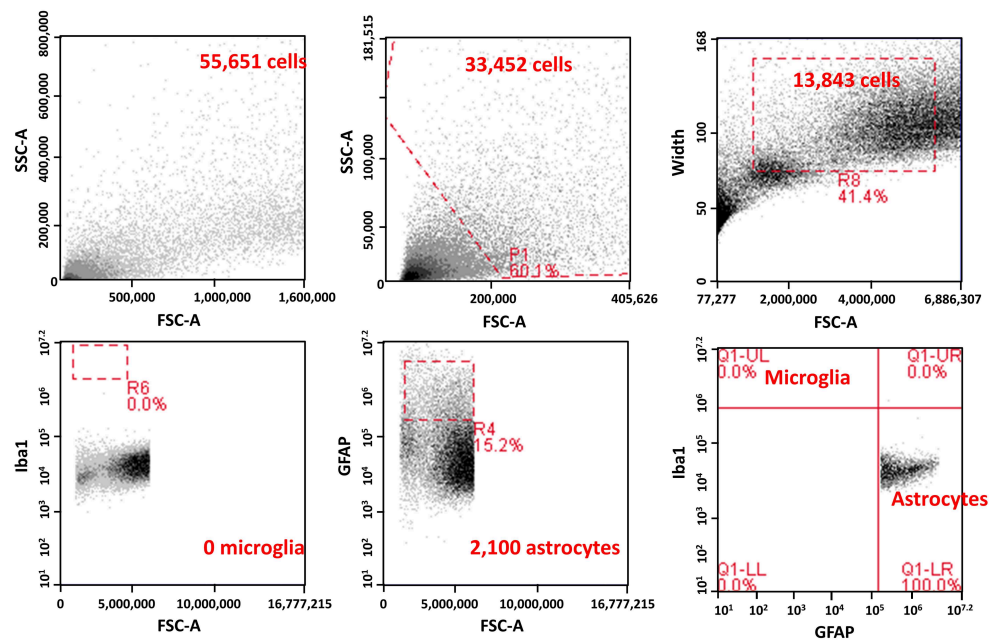
72.2 In the subsequent density plot of FSC-A x Width, the single cells are gated and selected, removing any clumps of cells from analysis.

72.3 Followed by this, the selected cells are analyzed for each of the cell-specific markers. First, microglia-specific marker Iba-1 was assessed by selecting an appropriate channel on the flow cytometer that is suited for the goat anti-rabbit 488 antibody detection. The

Iba-1 positive cells are expected to be seen in the top left quadrant of the FSC-A x Iba-1 density plot.

72.4 Similarly, in the FSC-A x GFAP plot, GFAP-positive cells (astrocytes) are expected in the top left quadrant.

72.5 By combining the gates from steps 72.3 and 72.4, both cell populations can be viewed on the same plot. Gates are applied as shown to determine astrocyte purity.



Protocol references

<https://medicine.yale.edu/immuno/flowcore/protocols/analysis/>

Albuquerque, C., et al. (2009). "Dissection, plating, and maintenance of cortical astrocyte cultures." Cold Spring Harb Protoc 2009(8): pdb prot5273.

Allen, J. W., et al. (2001). "Isolation of neonatal rat cortical astrocytes for primary cultures." Curr Protoc Toxicol Chapter 12: Unit12 14.

Foo, L. C., et al. (2011). "Development of a method for the purification and culture of rodent astrocytes." Neuron 71(5): 799-811.

Lin, J., et al. (2021). "Flow cytometry analysis of immune and glial cells in a trigeminal neuralgia rat model." Sci Rep 11(1): 23569.

Ni, M. and M. Aschner (2010). "Neonatal rat primary microglia: isolation, culturing, and selected applications." Curr Protoc Toxicol Chapter 12: Unit 12 17.

Orre, M., et al. (2014). "Acute isolation and transcriptome characterization of cortical astrocytes and microglia from young and aged mice." Neurobiol Aging 35(1): 1-14.

Schildge, S., et al. (2013). "Isolation and culture of mouse cortical astrocytes." J Vis Exp(71).

Souza, D. G., et al. (2013). "Characterization of adult rat astrocyte cultures." PLoS One 8(3): e60282.

Tabata, H. (2015). "Diverse subtypes of astrocytes and their development during corticogenesis." Frontiers in Neuroscience 9.

Zarei-Kheirabadi, M., et al. (2020). "Protocol for purification and culture of astrocytes: useful not only in 2 days postnatal but also in adult rat brain." Mol Biol Rep 47(3): 1783-1794.

Miyajima, A., et al. (2009). "Sexing of postimplantation rat embryos in stored two-dimensional electrophoresis (2-DE) samples by polymerase chain reaction (PCR) of an Sry sequence." J Toxicol Sci 34(6): 681-685.

Resources for BD Accuri C6 Flow Cytometer:

https://www.bdbiosciences.com/content/dam/bdb/products/instruments/flow-cytometers/research-cell-analyzers/accuri-c6/BD_Accuri_C6Flow_Cyto_Instrument_Manual.pdf

https://www.bdbiosciences.com/content/dam/bdb/products/instruments/flow-cytometers/research-cell-analyzers/accuri-c6/BD_Accuri_CSampler_Software_User_Guide.pdf