Aug 25, 2025

Brain Single Cell Isolation for Flow Cytometry

DOI
dx.doi.org/10.17504/protocols.io.kqdg3×8r7g25/v1

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Protocol Citation: Jhodi Webster, Ashley Harms, Vickie Yang, Aidan Miller 2025. Brain Single Cell Isolation for Flow Cytometry. **protocols.io https://dx.doi.org/10.17504/protocols.io.kqdg3x8r7g25/v1**

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

Created: October 13, 2023

Last Modified: August 25, 2025

Protocol Integer ID: 89268

Keywords: ASAPCRN, brain single cell isolation for flow cytometry, flow cytometry, brain single cell isolation, samples for flow cytometry analysis, flow cytometry analysis, single cells from mouse brain, single cell, intracellular cellular marker, mouse brain

Abstract

This protocol is used to isolate single cells from mouse brains and process these samples for flow cytometry analysis. The protocol can be optimized/altered to account for staining of intracellular cellular markers.

SETUP

1 Make reagents needed:
- Flow media: RPMI 1640 + 10%FBS + 1% Penicillin/Streptomycin + 1% L-Glutamine
- 10X Stock Enzyme: 1g Collagenase IV in 100mL serum-free media (RPMI 1640). Aliquot into 5mL and store at -20 °C until use. To use (working concentration) thaw on ice, QS to 38mL using serum-free media. Final concentration with 2mL sample is ~1mg/mL Collagenase IV.
- 90% Percoll: 18mL percoll + 2mL 10X PBS
- 70% Percoll: 7mL 90% + 3mL 1X PBS
- Staining buffer: 2% FBS in 1X DPBS without calcium and magnesium

2 Pre-heat water bath to 37C and pre-cool centrifuge to 4C. Label and set-up 6-wel plates, 50mL and 15mL conical tubes.

PROCEDURE

3 Isolate brain tissue using standard perfusion protocols and dissection techniques based on desired brain regions.

4 Place tissue in flat-bottom-6-well cell culture plate with 2mL flow media until tissue collection is complete.

5 Prepare enzyme and add 6mL working concentration to labeled 15mL conical tubes.

6 Mince isolated tissue with small scissors and transfer using pre-cut 1000µL pipette tips to conical tubes with enzyme.

7 Shake in water bath at 37C for 20 minutes: at 10 minutes, vortex and pipette up and down with Pasteur pipette to break up tissue then continue timer.

8 Transfer to 70µm cell strainers placed in 50mL tubes and smash tissue using the plunger of a 5mL syringe, rinsing with DPBS or flow media.

9 Spin at 1800RPM for 8min at 4C (brake on high)

10 Decant supernatant and re-suspend pellet in 7mL of flow media in 15mL conical tube.

11 Vortex cell suspension with 3mL 90% Percoll.

12 Inject 1.5mL of 70% Percoll under the 90% mixture SLOWLY. It is recommended to take-up 2mL of Percoll to inject, in order to prevent bubbles or disturbing the layer.

13 Spin at 1500RPM for 30min at 4C (no brake). DO NOT DISTURB GRADIENT.

14 Check for cells - should be suspended between pink and clear layer. Vacuum supernatant and fat to about 7mL line.

15 Transfer cells to a clean 15mL conical tube and fill rest of the way with PBS. DO NOT TAKE PELLET!

16 Vortex and spin at 1800RPM for 8min at 4C (brake on high).

17 Aspirate and leave 200uL liquid with pellet. Pipette up and down to break up pellet and transfer to round-bottom 96-well plate for staining. Label plate appropriately.
- Splenocytes: wells for single color control and unstained control (and optional: well for mastermind + splenocytes) - see steps below for splenocyte isolation
- Brain samples: mastermix only

18 Spin plate at 1500RPM for 5 min. Decant supernatant.

19 Re-suspend cells (only brain samples) in 100µL Fc Block (1:100 in staining buffer (FBS/PBS)) OR 100uL of staining buffer to splenocytes. Incubate on ice for 15 minutes, then add 100uL staining buffer on top for volume.

20 Spin plate at 1500RPM for 5min. Decant supernatant.

21 Make up antibody cocktail (mastermix) and single colour controls:

To make mastermix: add anitbodies at their specific concentrations to at least 500uL FBS/PBS (or based on how many samples you have)

To make single colour controls: use volume of 2% FBS/PBS that will allow for 1µL of each antibody. (i.e if CD45 is 1:200, use 200µL of FBS/PBS)

22 Re-suspend cells in 50µL antibody cocktail in staining buffer. Incubate 20-30 minutes on ice or at 4C.

23 Add 150uL staining buffer on top for 200µL total volume.

24 Spin 1500RPM for 5min. Decant supernatant.

25 Re-suspend cells in 200µL 2%PFA. Incubate on ice or at 4C.

26 Spin 1500RPM for 5min. Decant supernatant. Re-suspend in 200µL FBS/PBS.

27 For storage: foil and parafilm and place plate in 4C. Before running samples on flow cytometer, filter samples with a strainer cap into 5mL FACS tubes.

SPLENOCYTE ISOLATION

28 Isolate spleen from perfused mouse and place in 70µm strainer in 50mL conical tube.

29 Smash spleen with media and spin down at 1500RPM for 5min.

30 Decant media and add 5mL ACK lysis buffer into tube for 3-5 minutes. Neutralize with 10mL 1X DPBS.

31 Spon 1500RPM for 5min. Decant and resuspend in 10mL DPBS.

32 Spin 1500RPM for 5min. Aspirate and resuspend in 10mL flow media.