



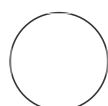
MAY 24, 2023

Protocols for processing of fresh murine tissues for flow cytometry

In 2 collections

Diana Rose E
Ranoa¹

¹University of Illinois at Urbana-Champaign



Diana Rose E Ranoa
University of Illinois at Urbana-Champaign

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.5qpvorb49v4o/v1

Protocol Citation: Diana Rose E Ranoa 2023. Protocols for processing of fresh murine tissues for flow cytometry. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.5qpvorb49v4o/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: Oct 27, 2022

Last Modified: May 24, 2023

PROTOCOL integer ID:
71864

ABSTRACT

To assess the immune cell composition immediately after 237 CAR treatment, we performed immunophenotyping by flow cytometry on samples from blood, spleen, pancreas, and ascites fluid recovered from ID8*Cosmc*-KO-bearing mice

1 Whole blood collected using heparinized capillary tubes (~70uL)

1.1 Incubate with 2mL of 1X ACK lysis buffer for 10 minutes then add 2mL RPMI+5%FBS

1.2 Centrifuge at 350xg for 5 minutes

1.3 Wash with 1mL PBS + 0.5% BSA buffer, then centrifuge at 350xg for 5 minutes. Perform twice.

1.4 Resuspend cells in 0.5mL PBS + 0.5% BSA buffer and store chilled on ice. Proceed to step

2 Spleens

2.1 Mechanically disrupt spleen through a sterile 70-µm nylon mesh filter using a 3 mL syringe

2.2 Washed with 20mL RPMI+5% FBS then centrifuge at 350xg for 5 minutes.

2.3 Incubate with 2mL 1x ACK lysis buffer for 10 minutes then add 2mL RPMI+5%FBS

2.4 Centrifuge at 350xg for 5 minutes

2.5 Wash with 1mL PBS + 0.5% BSA buffer, then centrifuge at 350xg for 5 minutes. Perform twice.

2.6 Resuspend cells in 1.0mL PBS + 0.5% BSA buffer (~100 million cells/mL) and store chilled on ice. Proceed to step 5.

3 Tumors up to 700mg in weight

3.1 Mince weighed tumor tissues using a razor blade on a 60mm petri dish.

3.2 Incubate for 20 min at 37 °C with 5mL digestion buffer consisting of 75 µg/mL Liberase DL (Sigma 5466202001) and 20 µg/mL DNase I (Sigma 4716728001).

3.3 Add 5mL trypsin-versene (1:1) to the slurry and pipette up and down for 2 minutes using a disposable 3 mL pipette.

3.4 Add 20mL RPMI+5% FBS and filtered through a 70um nylon mesh to generate single-cell suspension.

3.5 Centrifuge at 300xg, for 5 minutes at 4°C

3.6 Resuspend in 100uL PBS + 0.5% BSA buffer and store chilled on ice. Proceed to step 5.

4 Ascites

4.1 Collect ascites from euthanized mice at endpoint using a 20mL syringe with a 25G needle.

4.2 Centrifuge at 350xg for 5 minutes

4.3 Incubate cell pellet with 7.5mL of 1X ACK lysis buffer for 5 minutes then add 20mL RPMI+5%FBS

4.4 Centrifuge at 350xg for 5 minutes.

4.5 Wash with PBS + 0.5% BSA buffer, then centrifuge at 350xg for 5 minutes.

4.6 Resuspend cell pellet 4million cells/mL using freezing media (FBS + 10% DMSO) and store 1mL aliquots in -80oC until further use. If using right away, resuspend in PBS + 0.5% BSA buffer and store chilled on ice. Proceed to step 5.

5 Flow staining

- 5.1 Mix 100uL of dissociated cell suspension with 100uL of antibody cocktail containing 2x concentration of each of the following antibodies: anti-CD45_PerCP, anti-CD3_Pacific Blue, anti-CD8_BV605, anti-CD4_FITC, anti-CD11b_APC, OTS8-PE tetramer, and fixable viability dye eFluor 780.
- 5.2 Prepare single reagent stains using either blood or spleen
- 5.3 Incubate on ice for at least 30 minutes
- 5.4 Wash with 2mL PBS + 0.5% BSA buffer
- 5.5 Resuspend in 300uL PBS pH 7.4 containing 25uL of cell counting beads (Life Technologies C36950)
- 5.6 Perform flow cytometry on a BD LSRII with proper compensation of voltages using single-stained controls
- 5.7 Analyze fcs files with either FlowJo software or FCS Express

