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We use this protocol and it's working

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Protocols for processing of fresh murine tissues for flow cytometry

In 2 collections

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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