

Staining Mouse Peripheral Blood (CD45.2 donor into CD45.1 recipient)

Prepare 1.5 ml centrifuge tubes for blood collection:

150 µl Dextran T500 (2% in PBS)

150 µl PBS/EDTA/Heparin

>>> For 10 ml: 10 ml PBS + 160 µl .5M EDTA + tiny bit of heparin

Anesthetize mice using Isoflo or Avertin; bleed known CD45.1 and CD45.2 mice for controls

Take PB in heparinized capillary tube (~ 65µl)

For engraftment analysis: 1 tube

For lineage analysis: 3 tubes

Let stand at RT for 20-30 minutes (no longer than 30 minutes)

After RBC have settled, take off top phase containing WBC and put into new tube

Spin down supernatant: 2500 rpm x 5 minutes

Prepare lysis buffer fresh:

For 10 ml: 9ml 0.16M NH₄Cl + 1ml 0.17M Tris-Cl pH 7.65

Resuspend pellet in lysis buffer and let stand at RT for 10 minutes

For engraftment analysis: resuspend in 1ml

For lineage analysis: resuspend in >= 1.5ml

Spin down: 2500 rpm x 5 minutes, and resuspend in HBSS+

(can leave o/n at 4° here, or after block or stain)

Re-spin, and prepare blocking solution:

33% Mouse serum 67% HBSS

Resuspend pellet in 100 µl blocking solution, and block for 15 min

Prepare antibody staining cocktails

Engraftment: Mix equal amounts CD45.2 FITC and CD45.1-PE (1 µl each / sample)

Lineage: Prepare 4 cocktails, diluting antibody 1.25:100 in 100µl per sample

A: CD45.2 FITC and CD45.1 PE

B: CD45.2 FITC, Mac1-PE and Gr1-PE

C: CD45.2 FITC, CD4-PE and CD8-PE

D: CD45.2 FITC, and B220-PE

Mix CD45.1 and CD45.2 cells together, and divide into 5 controls (40 µl each)

No staining No antibody OR PI

PI only Resuspend in PI containing media

PE comp Stain with B220-PE, wash and resuspend in PI 1:100

FITC comp Stain with B220-FITC, wash and resuspend in PI 1:100

Mixed control Stain with CD45.2-FITC AND CD45.1-PE, resuspend in PI 1:100

Divide samples (in blocking sol.) into 4 tubes ("Sample A," "Sample B," "Sample C," "Sample D")

Add 100 µl of appropriate antibody to each tube and stain for 15 minutes at 4°

Wash with 10-20 volumes of HBSS+ and spin down (2500 x 5)

Resuspend in 300 µl of HBSS+ with PI 1:100, and analyze by flow