Spleen Immunophenotyping: Sample Preparation

Protocol for processing mouse spleens
into single cell suspension

Reagents & Buffers

1. HBSS (Invitrogen14170-138)
2. Enzyme Buffer (PBS (+Mg/+Ca), 2% FCS, 10 mM HEPES, Collagenase 1mg/ml (Roche, 11088858001), DNAse 0.1 mg/ml (Sigma, DN25))
3. RBC lysis solution (eBiosciences 00-4300-54, made up to 1x with ddH_2O)
4. FACS buffer (PBS (-Mg/-Ca), 0.5% FCS, 2 mM EDTA, 10 mM HEPES)
5. Stop buffer (PBS (-Mg/-Ca), 0.1 M EDTA)
6. PBS (-Mg/-Ca)

Materials

1. Miltenyi C Tubes
2. 15ml tubes
3. 30 µm CellTrics filters (Partec, 04-0042-2316)
4. Dispensing troughs for multichannel pipetting
5. 96-well 350 µl Polypropylene V-bottom plates (BD Falcon 353263)

Equipment

1. GentleMACS tissue dissociator (Miltenyi)
2. 37°C water bath
3. Centrifuge

Samples are shipped as dissected spleens in 1.7 ml tubes containing HBSS on ice from WTSI to KCL (approximately 2 hours by courier) and processed on the same day.

1. Prepare buffers and antibody master mixes (see staining protocol) beforehand. Label plates for staining.
2. Fill one C-tube per spleen with 3 ml enzyme buffer.
3. Dissect spleens from fat. Transfer cleaned spleens into C-tubes.
4. Run C-tubes on program Spleen-02 on a Miltenyi GentleMACS dissociator.
5. Incubate at 37°C for 30 minutes in a water bath.
6. While spleens are incubating, prepare required number of 15 ml tubes with 30 μm CellTrics filters. Pipette 6 ml FACS buffer through the filters.
7. After removing spleens from water bath, run C-tubes on program Spleen-03 on a Miltenyi GentleMACS Dissociator.
8. Add 300 μl stop buffer to each tube.
9. Filter contents of C-tubes into 15 ml tubes. Tap gently and discard filters.
10. Centrifuge for 5 minutes at 400×g at 8°C and check for cell pellet.
11. Discard supernatant and resuspend pellet in 1 ml FACS buffer.
12. Pipette 50 μl of each sample into prepared 96-well V-bottom plates.
13. Centrifuge plates for 1 minute at 800×g at 8°C.
14. Resuspend in 50 μl of room temperature 1x RBC lysis buffer. Incubate for 90 seconds. Top up with 150 μl FACS buffer.
15. Centrifuge plates for 1 minute at 800×g at 8°C.
16. Cells are now in single cell suspension on plates and ready for staining (see staining protocol).