Mesenteric Lymph Node Immunophenotyping: Sample Preparation

Protocol for processing mouse mesenteric lymph nodes
into single cell suspension

Reagents & Buffers

1. HBSS (Invitrogen14170-138)
2. Preparation buffer (PBS (+Ca/+Mg), 2% FCS, 10 mM HEPES)
3. Enzyme buffer (PBS (+Ca/+Mg), 2% FCS, 10 mM HEPES, Collagenase 1.5 mg/ml (Roche 11088858001), DNAse 0.1 mg/ml (Sigma DN25))
4. RBC lysis solution (eBiosciences 00-4300-54, made up to 1× with ddH_2O)
5. FACS buffer (PBS (-Mg/-Ca), 0.5% FCS, 2 mM EDTA, 10 mM HEPES)
6. Stop buffer (PBS (-Mg/-Ca), 0.1 M EDTA)
7. PBS (-Mg/-Ca)

Materials

1. 1.7 ml microfuge tubes
2. 15 ml 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. 37°C water bath
2. 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 required number of 1.7 ml microfuge tubes with 200 µl preparation buffer
3. Dissect lymph nodes from membrane and fat. Put lymph nodes into prepared 1.7 ml tubes.
4. Rupture lymph nodes with mini pestles.
5. Add 400 µl enzyme buffer.
6. Incubate at 37°C for 20 minutes in a water bath.
7. While MLN are incubating, prepare required number of 15 ml tubes with 30 µm CellTrics filters.
8. After removing MLN from water bath, add 60 µl stop buffer.
9. Filter contents into 15 ml tubes.
10. Rinse out microfuge tubes with 1 ml FACS buffer and also filter into 15 ml tubes.
11. Wash filters with 5 ml FACS buffer. Tap gently and discard filters.
12. Centrifuge for 5 minutes at 400xg at 8°C and check for cell pellet.
13. Resuspend in 500 µl FACS buffer. Remove any visible floating fat or debris.
14. Pipette 165 µl of each sample into prepared plates.
15. Cells are now in single cell suspension on plates and ready for staining (see staining protocol).