Epidermis immunophenotyping

Protocol for staining of epidermal sheets and preparation of slides

Reagents & Buffers

1. Staining buffer (PBS (-Mg/-Ca), 2% FCS)
2. PBS
3. Anti-mouse Vγ3 TCR FITC (clone 536, BD 553229), use 1:500
4. Anti-mouse I-A/I-E-A647 (clone M5/114.15.2, Biolegend 107618), use 1:500
5. Anti-mouse CD45 eFluor450 (clone 30-F11, eBioscience 48-0451-82), use 1:200
6. Prolong Gold solution (New Enlgand Biolabs 9071S), defrost before use
7. Nail polish

Materials

1. 1.7 ml microfuge tubes
2. Forceps (Dumont #7)
3. Frosted slides (VWR 631-0108)
4. Coverslips (VWR 631-1379)

Equipment

1. Table top shaker
2. Stereomicroscope

Samples are shipped as fixed epidermal sheets in 1.7 ml tubes containing PBS / 0.5 mM sodiumazide on ice from WTSI to KCL (approximately 2 hours by courier) and processed the same week (samples can be kept in this solution for a few months if restaining is required).

1. Prepare one 1.7 ml tube with 180 µl staining buffer per sample.
2. Put a large drop of staining buffer on a slide. Under the stereomicroscope, place an epidermal sheet on top and unfold the sheet using forceps.
3. Transfer the epidermal sheet into the prepared 1.7 ml tube, making sure it does not fold up again and is exposed to the solution. Use a pipette tip to unroll the sheet again if necessary.
4. Incubate at room temperature for 60 minutes in order to block unspecific antibody binding sites.
5. Prepare the antibody master mix: per sample use 18.2 µl staining buffer, 0.4 µl anti-Vγ3 FITC, 0.4 µl anti-MHC II AF647 and 1 µl anti-CD45 eFluor450.
6. Add 20 µl antibody mix to each tube and incubate for 75 minutes at 37°C and 450 rpm on a table top shaker.
7. Wash samples 4 times for 3-5 minutes with 0.5 ml PBS. Use a pipette to remove and add liquid. Store in the fridge until slide preparation.
8. To prepare slides set up a stereomicroscope with a table top light and fibre optics illumination. Using a stereomicroscope helps to identify the dermal side of the epidermal sheet and stretch the epidermal sheet on the slide efficiently (hair side down, if no hair is present this side looks rougher).
9. Transfer the epidermal sheet onto a labelled slide and use forceps to stretch it out dermal side down
10. Place a drop of Prolong Gold solution on the epidermal sheet and cover with a coverslip. Gently press down the coverslip with the forceps, squeezing out any bubbles.
11. Seal the coverslip with nail polish. The slide is now ready to be imaged.