Cancer Biology Summer School 2025

Flow Cytometry

Requirements:

- Compensation beads
- Fluorescent-conjugated antibodies – **anti-CD4 PE** and **anti-CD8 FITC**
- Round-bottom polystyrene (FACS) tubes
- FACS buffer (PBS with 0.5% BSA and 0.05% Sodium Azide – filter sterilised)

Product and supplier's details of all reagents provided in the appendix.

I. Sample preparation

1. Label 4 tubes -- for unstained beads, beads stained with CD4 PE, beads stained with CD8 FITC, and beads stained with both antibodies. You could label tubes 1-4 or A-D and note down full names (unstained etc.) in your notebook.

2. Add 500 µl of FACS buffer to each FACS tube (use P1000).

3. Mix compensation beads in their original container by pulse-vortexing.

4. Add one drop of compensation beads to each FACS tube.

5. Centrifuge at 1400rpm at 4°C for 5 minutes.

6. In the meantime, make up fluorescent-conjugated antibodies (CD4 PE and CD8 FITC) in FACS buffer at 1:100 dilutions. To do this, prepare three Eppendorf tubes. Label two Eppendorf tubes with antibody names and add 198 µl of FACS buffer to each tube. Add 2 µl of appropriate antibody to each tube. To the third tube, add 196 µl of FACS buffer. Add 2 µl of each antibody (making up 200 µl total). Pipette the contents of each tube up and down five times to mix (use P200).

	CD4 PE dilution	**CD8 FITC dilution**	**CD4 PE and CD8 FITC dilution**
FACS buffer (µl)	198	198	196
Antibody (µl)	2	2	2 + 2

7. Carefully remove FACS tubes from the centrifuge. Remove the supernatant (FACS buffer) by decantation, i.e. turn each tube upside down in one smooth motion and pour the supernatant down the sink.

8. Add 100µl of the single fluorescent-conjugated antibody at working dilution that you have just prepared to appropriately labelled tubes. Do not add anything to the sample of unstained beads at this point.

9. To prepare the sample of unstained beads, take 100 µl of plain FACS buffer (no antibody, from the original stock) and add this to the remaining tube. You should have following samples

Sample	Unstained	CD4 PE stained	CD8 FITC stained	Stained with both antibodies
Resuspended in	100 µl of plain FACS medium (no antibodies)	100 µl of CD4 PE 1:100 dilution from Step 6	100 µl of CD8 FITC 1:100 dilution from Step 6	100 µl CD4 PE and CD8 FITC 1:100 dilution from Step 6

10. Vortex to mix beads and antibody well, wrap in aluminium foil to protect from light, and incubate in the fridge at 4°C for 20 minutes.

11. After the incubation, add 2 ml FACS buffer to each tube and centrifuge using the same setting as in Step 5.

12. Remove supernatant as before and resuspend beads in 400ul FACS buffer.

13. Later vortex for a few seconds immediately before using the flow cytometer. This will give positive and negative antibody staining peaks.

II. **Running samples on the flow cytometer** – as per guidance given by the instructors

III. **Results analysis & discussion**

APPENDIX

- Compensation beads (Invitrogen, UltaComp eBeads, Cat. No. 01-2222-41)

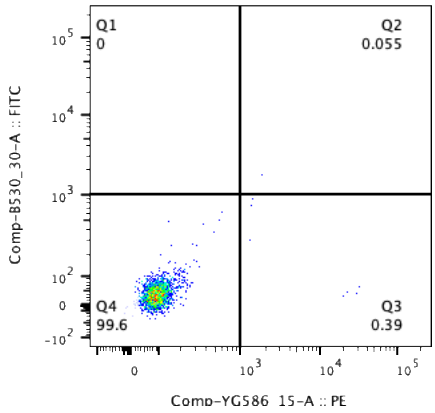
- Fluorescent-conjugated antibodies – **anti-CD4 PE** (BioLegend, PE anti-mouse CD4 Antibody, Cat. No. 100407/8) and **anti-CD8 FITC** (Biolegend, FITC anti-mouse CD8a Antibody, Cat. No. 100705/6).

- Round-bottom polystyrene (FACS) tubes (Fisher Scientific, 5 mL Falcon Round-Bottom Polystyrene Test Tubes, 12 x 75 mm style, Cat. No. 10585801)

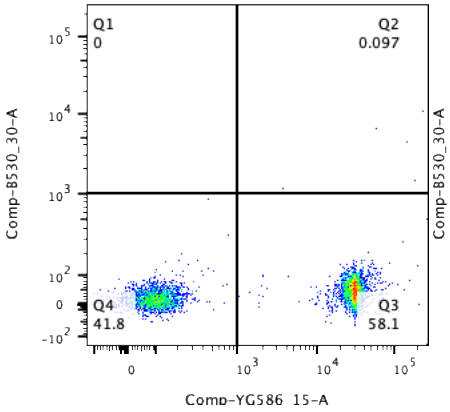
- FACS buffer (PBS with 0.5% BSA and 0.05% Sodium Azide – filter sterilised)
    - PBS (Thermofisher Scientific, Gibco PBS Tablets, Cat. No. 18912014)
    - BSA (Sigma-Aldrich, Bovine Serum Albumin, Cat. No. 9048-46-8)
    - Sodium Azide (Sigma-Aldrich, Reagent Plus Sodium Azide, Cat. No. 26628-22-8)

Sample results

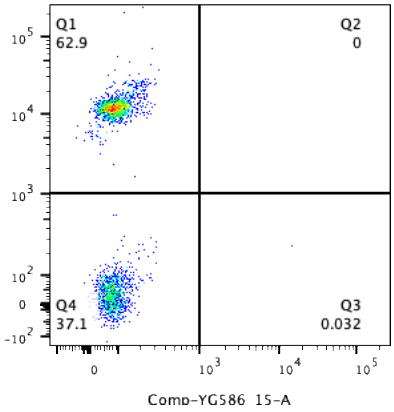
Unstained Control



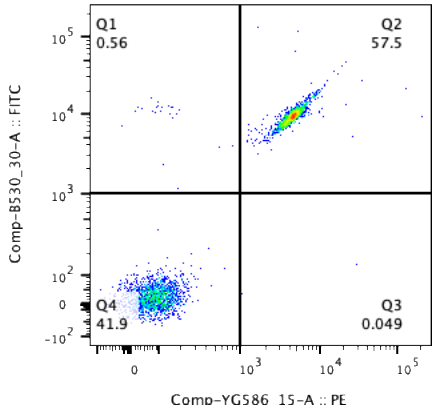
Single stained-CD4



Single stained-CD8



CD4 & CD8 double stained



CD8; FITC

CD4; PE