Flow Cytometry Prep for 24 Samples

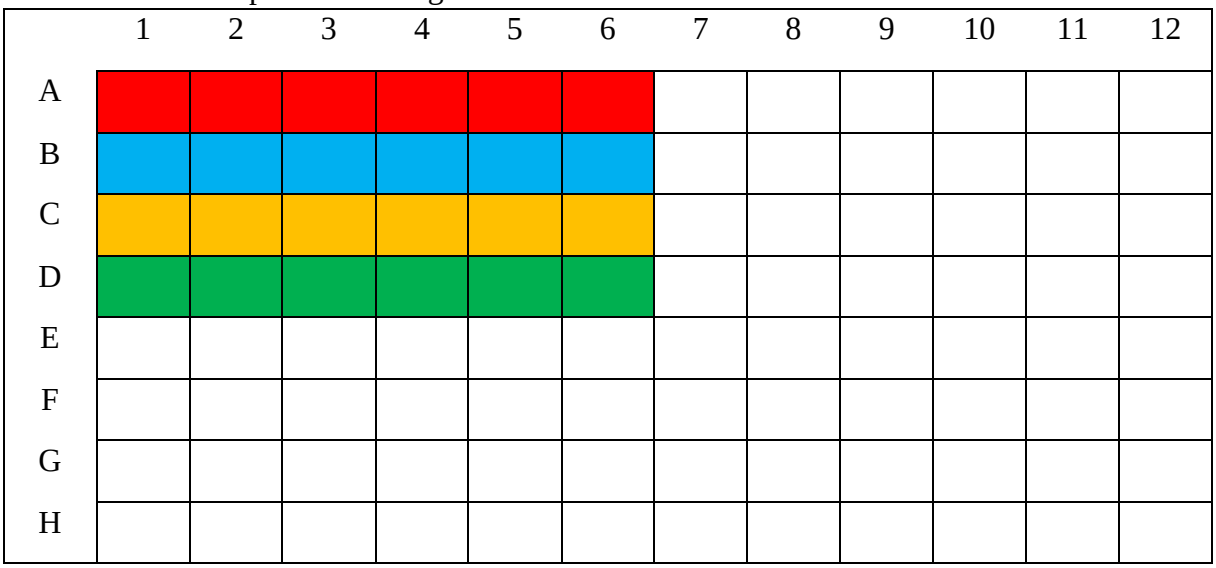
- Collect CT on incomplete RPMI
- Open up CT and remove all debris. We just want to strain the small papillae. Use gentle force
- Strain 1 CT/pen, mucosa side down, with 3-5 mL of RPMI through the cell strainer
- Take all of the product into a 15 ml conical tube and add RPMI so all have 6 mL volume
- Centrifuge the cells **@ 4,000 rpm** for 6 min at 10 C with break
- Discard the supernatant, keep the pellet. Add 1 mL incomplete RPMI to pellet and mix
- Repeat centrifuge @ 4000 rpm for 4 min
- Discard the supernatant. Add 200 µL RPMI then mix the pellet 3x
- Cells are ready for flow
- Turn on the machine. Do not forget to clean it once a week or Dr. Revathi will be upset

CD4+CD8+ Calculations

- Can be prepared in advance then put into the flow plate the day before use
- In a foil covered 15 mL conical tube, add 2465 µL wash buffer and 25 µL mouse serum then invert several times to mix
- Turn off the lights
- Vortex your anti-CD4 (CT-4)(FITC) CD4+ then add 10 µL to the tube
- Vortex your CD8a-PE then add 8 µL to the tube
- Mix all by inversion

CD4+CD8+ Plating

- In darkness using flow plates, add 85 µL of your prepared CD4+CD8+ to each well of the 96 well plate following the scheme below



Each color represents a treatment group. Leave 7-12 empty for dilutions. Leave E-H empty for next week's flow. Reuse the plate. Reuse the trough (wash first)

- Seed 15 µL to each well of RPMI cells. Call Dr. Revathi before you do this step
- Mix with buffer using a multichannel pipette
- Cover and incubate at 4C for 20 min
- You are ready for washing and flow

T-reg Calculations

- Prepare and put buffer in plates the day before then refrigerate
- Turn the lights off. In a conical 15 mL tube, combine 2500 µL running buffer with 48 µL CD25 (vortex before addition) and26 µL mouse serum. Vortex

T-reg Plating

- In darkness add 85 µL of your prepared Treg buffer to each well using the same scheme as above
- Add 15 µL strained cells to each well
- Incubate at 4C for 50 min
- Mix 250 µL running buffer with 8 µL CD4 FITC and add 10 µL/well DOWN THE SIDE of each well
- Mix with a multichannel pipette
- Incubate at 4C for 20 min
- Ready for washing and flow