Flow Cytometry for CD4+ CD8+ from CT

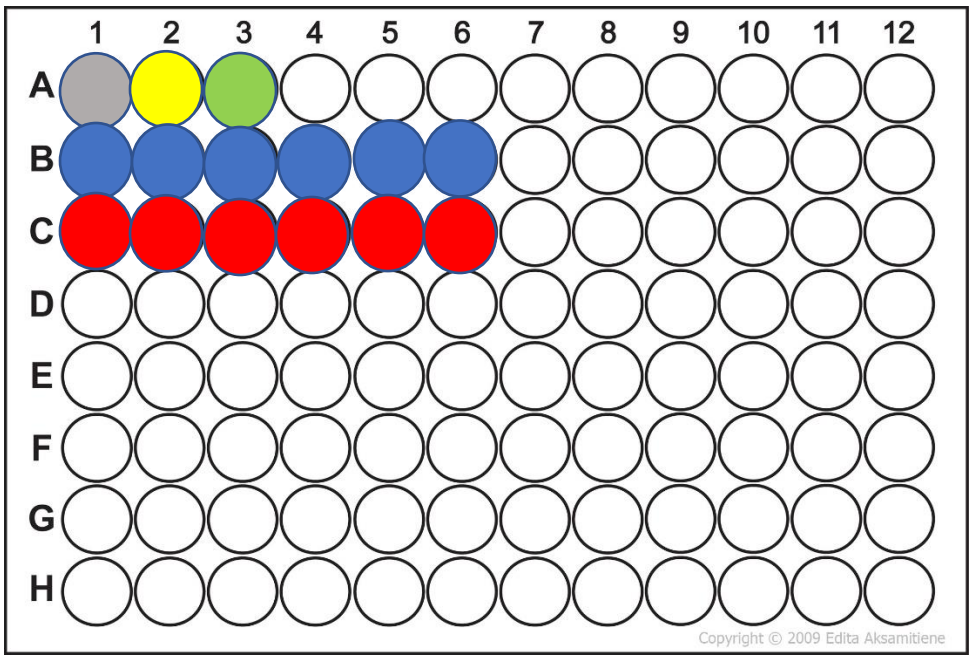
1. Flow cytometry running buffer (wash buffer)
   - Dissolve 5g of BSA in 950 mL of PBS while stirring
   - Add 4 mL of 0.5M EDTA
   - Adjust pH to 7.4
   - Seal with a paraffin and store at 4°C
2. See CT processing protocol briefly, strain CT and layer on Ficoll for T-lymphocyte enrichment
3. Seed 100 µl of 10^6 cells of CT per well
4. Add 50 µl of each CD4 and CD8 diluted in 1:100 (Note this time we are keeping CD4 and CD8 separate and using 100 µl of cells)
5. Incubate for 30 min at 4°C
6. Wash twice (750 rcf at 10 C for 4 min) with running buffer and resuspend the cells in 200 µl of wash buffer (Covered with foil and keep on ice)

Today's calculations:

- **Add 10** µl of CD4-PE to 990 µl of wash buffer
- **Add 10** µl of CD8-FITC to 990 µl of wash buffer

For T regs (CD4+CD25+)

1. Seed 100 µl of 10^6 cells per well
2. Add 50 µl of CD25-PE (1:100) and incubate at 4°C for 40 minutes
3. Add 50 µl of CD4-FITS (1:100) and incubate at 4°C for 20 min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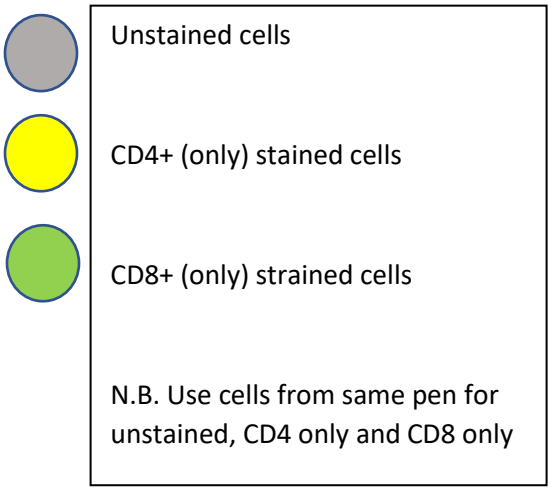
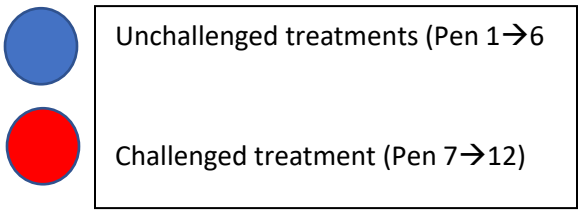
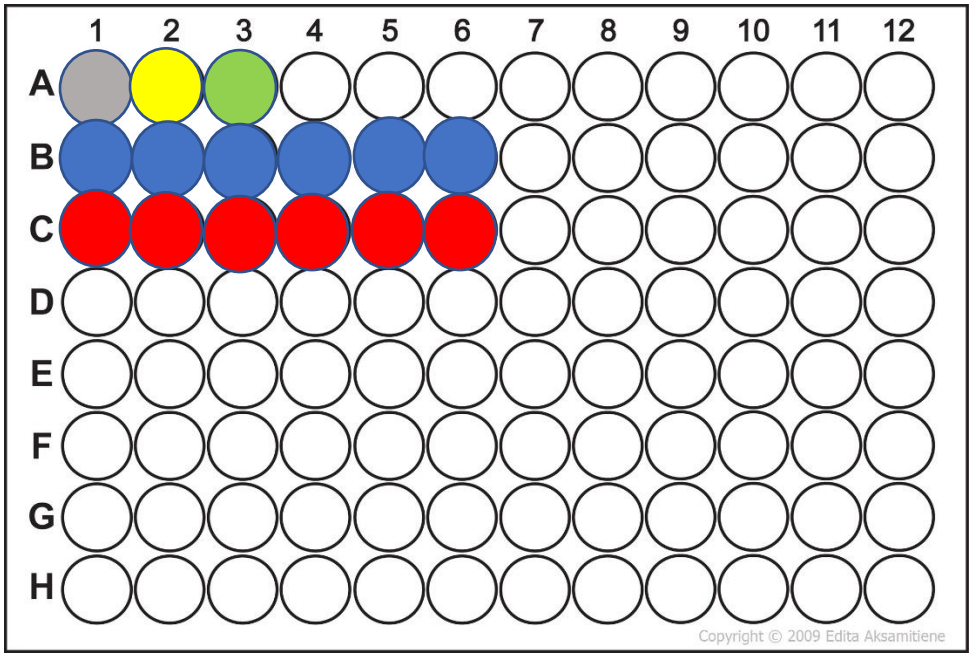
CD4+/CD8+ plan


Unstained cells
CD4+ (only) stained cells
CD8+ (only) strained cells
N.B. Use cells from same pen for unstained, CD4 only and CD8 only


Unchallenged treatments (Pen 1→6
Challenged treatment (Pen 7→12)



CD4+/CD8+ plan

	Unstained cells
	CD25-PE (only) stained cells
	CD4-FITC (only) strained cells
	N.B. Use cells from same pen for unstained, CD25 only and CD4 only

	Unchallenged treatments (Pen 1→6
	Challenged treatment (Pen 7→12)