Flow Cytometry CD4+/CD8+

- Strain 3 CT/Cage on 3 mL of RPMI
- Take 1 mL of the following into a 2 ml tube and add 1 mL of RPMI
- Centrifuge the cells **@ 6,000 rpm** for 10 min at 10 C
- Discard the supernatant and as Revathi for the amount of wash buffer to add
- Seed cells at 15 µL per well in flat bottom ELISA plates
- Add CD4+ and CD8+ solution to all cells
- Keep A1 Cells only, A2 CD4+ only, and A3 CD8+ only
- Incubate for 30 min
- Centrifuge the plate **@ 750 rcf** 10 C for 4 min
- Add 200 µL wash buffer
- Repeat centrifugation
- Resuspend cells in 200 µl and tale 10 µl into a new 200 µl of wash buffer
- Cells are ready to flow

CD4+CD8+ calculations

- Add 1,500 µl of wash buffer in 2 mL tubes
- Take out 18 µL
- Add 7.5 µL of CD4+ and 7.5 µL of CD8+ (1:200)
- Add 3 µL of Mouse IgG to block (1:500)

CD4+ only

- Add 200 ul of wash buffer
- Take out 1 µL
- Add 1 µL of CD4+ only

CD8+ only

- Add 200 ul of wash buffer
- Take out 1 µL
- Add 1 µL of CD8+ only