**FACS T cell differentiation/markers**

1. Collect cells (1-2 mln cells) on ice
2. Spin down for 5 min, 150g @ 4⁰C
3. Remove supernatant
4. Wash cells in 5 ml ice-cold PBS and resuspend
5. Spin down for 5 min, 150g @ 4⁰C
6. Remove supernatant
7. **Unstained ALLSIL cells**
   1. Resuspend in 250 µl of FACS buffer
8. **ALLSIL cells samples stained with Aqua (live/dead stain) + 8-color staining** 
   1. Stain with Aqua 1:1000 *(Freezer -20°C, Elisa tools,10 min 4°C)*
   2. Spin down for 5 min, 150g @ 4⁰C
   3. Remove supernatant
   4. Wash cells in 5 ml ice-cold PBS and resuspend
   5. Spin down for 5 min, 150g @ 4⁰C
   6. \*For Aqua compensation control = resuspend in 250 µl of FACS buffer (4°C)
   7. Add 8-color staining mix in FACS buffer (50 µL) – *(Fridge 4°C colored boxes)*
      1. TCRαβ-FITC 1:100
      2. CD1a-PE 1:200
      3. CD7- PerCP-Cy5.5 1:100
      4. CD5-PE-Cy7 1:200
      5. CD4-APC 1:200
      6. CD3-AP-CH7 1:200
      7. CD8-HzV450 1:200
   8. Incubate for 30 min at 4°C in DARK
   9. Add 1 ml of FACS buffer
   10. Spin down for 5 min, 150g @ 4⁰C
   11. Remove supernatant and resuspend in 250 µl of FACS buffer (4°C)
9. **Beads with different antibodies as compensation controls** 
   1. Vortex beads (Fridge 4°C – bag with blue and white cap tube)
   2. Put 2 drops of beads in 800 µl of FACS buffer
   3. Divide over 8 tubes and vortex
   4. Add 0.5 µl of appropriate antibody to each tube and vortex
      1. TCRαβ-FITC
      2. CD1a-PE
      3. CD7- PerCP-Cy5.5
      4. CD5-PE-Cy7
      5. CD4-APC
      6. CD3-AP-CH7
      7. CD8-HzV450
      8. Unstained beads (no antibody added)
   5. Incubate for 15-30 min at RT in DARK
   6. Add 1 ml of FACS buffer
   7. Spin down for 5 min, 150g @ 4⁰C
   8. Remove supernatant and resuspend in 250 µl of FACS buffer (4°C)