**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)