

November 23-24th, 2021

HEU: Ex-vivo $\gamma\delta$ CBMC panels #12 & ILT novo 5

| Specimen | Status | Location | Conc | Date | Tasks | Volume | Ly | Ly+Mon | Total | 1E+6 | 1.5E+6 |
|----------|--------|----------|------|------|-------|--------|----|--------|-------|------|--------|
| Inf 212 | Heu-lo | | | | | | | | | | |
| Inf 187 | HEU-hi | | | | | | | | | | |
| | | | | | | | | | | | |
| | | | | | | | | | | | |

4*1.2 = 4.8 μ l of hCOB 1:9
20 μ l of hMR2 3:27

Tetramers @ 8:36pm Fuel... \checkmark phone displayed wrong alarm tag...
→ at 9:03 same time? → 27. thinks google

too f late now 27 minutes

~~maybe~~ maybe will have less non-specific. ~~xxxx~~
(or halved % of real cells...)

→ 37°C @ 9:22 → 9:52 (← (+ new $\gamma\delta$ CR3 etc!))
2 ml FBS \checkmark

4°C @ 10:05 → 10:35pm!

no rbc lysis...
→ lysed for 3ish minutes & washed w/ FBS again

Fix Perm @ 11:03 → 13 → 23

Intra @ 23:44pm

Done @ 00:35 am

22:12 Perm wash
→ 22 → 32
2nd wash \checkmark

| # | Filter | Single color (u) | Ref cti | Fluorochrome | Marker | During stimulation | Tetramer (RT) for 40 min | 37°C (RT) for 30min | Surface staining 40C for 30min | Lysing then Cytofix/Perm | RT for 40min Universal intra mix (10x diluted) | |
|--------------------|--------|------------------|---------|--------------|-----------------|-----------------------|--------------------------|---------------------|--------------------------------|--------------------------|--|--|
| 1 | UV2 | 1.25 | Cells | C | BUV395 | CD62L (DREG-56) | | | | | | |
| 2 | UV7 | 1.25 | Cells | C | BUV496 | CD38 (BPA-18) | | | | | | |
| 3 | UV9 | 1.25 | Cells | C | BUV563 | CD69 (FNS0) | | | | | | |
| 4 | UV10 | 1.25 | Cells | C | BUV615 | CCR4 (LG1) | | | | | | |
| 5 | UV11 | 1.25 | Cells | C | BUV651 | Vα2 (B6) | | | | | | |
| 6 | UV14 | 1.25 | Cells | C | BUV737 | CD38 (LG6/CCR3) | | | | | | |
| 7 | UV16 | 1.25 | Cells | C | BUV805 | CD4 (SK3) | | | | | | |
| 8 | V1 | 1.25 | Cells | C | BUV421 | CD127 (A01905) | | | | | | |
| 9 | V3 | 1.25 | Cells | C | Pacific Blue | CD14 (M5E2) | | | | | | |
| 10 | V5 | 1.25 | Cells | C | Pacific Blue | CD19 (H1B19) | | | | | | |
| 11 | V7 | 1.25 | Cells | C | BUV480 | CD161 (FNA631) | | | | | | |
| 12 | V10 | 1.25 | Cells | C | BUV510 (dim) | CD45RA (H1100) | | | | | | |
| 13 | V13 | 1.25 | Cells | C | BUV605 | CD56 | | | | | | |
| 14 | V14 | 1.25 | Cells | C | BUV711 | Hsu CCR7 (separately) | | | | | | |
| 15 | V15 | 1.25 | Cells | C | BUV750 | IFNγ (B27) | | | | | | |
| 16 | B2 | 1.25 | Cells | C | BUV786 | CCR6 (11A9) | | | | | | |
| 17 | B3 | 1.25 | Cells | C | Alexa488 | hCD1d | | | | | | |
| 18 | B4 | 1.25 | Cells | C | Spark blue 550 | CD3 (SK7) | | | | | | |
| 19 | B6 | 1.25 | Cells | C | PE | NKG2D | | | | | | |
| 20 | B8 | 1.25 | Cells | C | PE-CF594 | CD26 (M-A261) | | | | | | |
| 21 | B10 | 1.25 | Cells | C | PE-CF594 | CD25 (M-A251) | | | | | | |
| 22 | B13 | 1.25 | Cells | C | PerCP-Cy5.5 | ITIA (MA811) | | | | | | |
| 23 | R1 | 1.25 | Cells | C | PE-wo/70 | PD1 | | | | | | |
| 24 | R2 | 1.25 | Cells | C | APC | IL-4 | | | | | | |
| 25 | R4 | 1.25 | Cells | C | Alexa Fluor 647 | hMIR1 50P-RU | | | | | | |
| 26 | R6 | 1:2500 | Cells | C | APC-R700 | CD107a (H4A3) | | | | | | |
| 27 | R7 | 1:3 | Cells | P | Zombie NIR | Viability | | | | | | |
| 28 | R8 | 1:3 | Cells | P | APC/Fire 750 | CD27 (O323) | | | | | | |
| 29 | R8 | 1:3 | Cells | C | APC/Fire 810 | CD38 (H17) | | | | | | |
| Total volume | | | | | | 7.6 | | | | | | |
| unstimulated Cells | | | | | | | | | | | | |
| Z7 color CBMC | | | | | | | | | | | | |

Brilliant Stain: 50
Draw Volume: 57.1

57.1

62.6

52.3

Protocol

| | |
|-------------------------------|---------------|
| Aliquot cells | |
| PMMA-ionomycin stimulation | |
| Golgi blockers | |
| PBS 2ml | 1400rpm, 6min |
| Live/Dead @RT 15min | |
| PBS-FBS 2ml | 1400rpm, 6min |
| Tetramers @RT 40min | |
| PBS-FBS 2ml | 1400rpm, 6min |
| Surface | CCR4 CD26 |
| 37°C 30min | CCR3 CD27 |
| | CD161 CD38 |
| PBS-FBS 2ml | 1400rpm, 6min |
| Surface mix @ 40C 30min | |
| Lysing solution 300-500ul | |
| PBS-FBS 2ml | 1400rpm, 6min |
| Cytofix/perm @ 40C 20min | |
| wash twice PermWash 1 ml | 1500rpm, 6min |
| Intra Staining @ RT for 40min | |
| wash once w/ 2ml | 1500rpm, 6min |
| Resuspend 100 ul PFA-PBS | |

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DTR - ILT - 1124 21

FSC 90 SSC 300 Ssc B 142

(not a monocyte setup obvious ly)

→ cap CCR3

clone VD2

4SR+ act

better

111021

CCR4 custom

NG2D

4SR+ custom

CCR6

CCR6 stim

check 26 ✓

→ VD2 1

S6 → 111021 not act

NG2D → 1121

PD1 → 1121

IL4 → TNF α

activated CCR4/6

→ not this one

unstained includes downregulated

CD3 unmixing...

→ 4SR+

CCR4 × CCR6

CCR6 ✓

Build NG2D

26

Jiang Hu forgot to shut down the

21-color

& no users on LSR after me... so just running

