October 1-2nd, 2021

HEU: Ex-vivo γδ CBMC panels #2 & MAIT NKT surface/ P2

| Specimen | Status | Location | ~~Cone~~ Date | ~~Date~~ Conc | Tasks | Volume | Ly | Ly+Mon | Total | 3E+6 | 1.5E+6 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Inf055-0 a-1 (8) | HU RVQ4 | Box 3A E4, E5 | 2018/ 10/15 | | | 4 | 2.84 | 3.95E6 | 11.36 | 750µl +360 | 528µl |
| Inf374-4 a-1 (16) | HEU-L SV45 | Box 12A A7, A8 | 2020/ 03/20 | | | 4 | 3.52 | 4.67E6 | 14.08 | 650ul +50ul | 426µl |
| Inf313-7 a-1 (12) | HEU-H 9NFC | Box 9A Row I1, I2 | 2019/ 10/18 | | | 4 | 1.58 | 2.71E6 | 6.32 | | 950µl |
| ND006 | Adult | | 01/27/21 | | | 3 | 4.0 | 5.08E6 | 12.18 | (1.5E+6) 300ul +750 Rio | 370µl |
| | | | | | | | | | | | |

| | Ex vivo Gd-p4b | Surface µL | x 3 | Intra µL | x 3 |
|---|---|---|---|---|---|
| BV421 | PD1 | 2 | 6 | | |
| BV510 | Aqua L/D | 1:500 | | | |
| BV650 | CD56 | 1.8 | 5.4 | | |
| Alexa 488 (FITC) | Perforin (dG9) | - | - | 3 | 9 |
| PerCPeF710 | CD3 | 1.5 | 4.5 | | |
| PE | GZMb | - | - | 1.5 | 4.5 |
| PE Dazzle | | | | | |
| PE Vio770 | NKG2A | 0.6 | 1.8 | | |
| APC | Vδ2 | 1 | 3 | | |
| APC Fire750 | CD16 | 1 | 3 | | |
| **20.5 µl/rxn;** | PBS | 12.6 | 37.8 | 16.0 | 52.5 |

| | Ex vivo Gd-CK PMA | Surface µL | x 6 | Intra µL | x 6 |
|---|---|---|---|---|---|
| BV421 | PD1 | 2 | 12 | | |
| BV510 | Aqua L/D | 1:500 | | | |
| BV650 | CD27 | 1.5 | 9 | | |
| FITC | Vδ2 | 1.2 | 7.2 | 0.2 | 1.2 |
| PerCPeF710 | CD3 | 1 | 6 | 0.2 | 1.2 |
| PE | CD56 | 0.5 | 3 | | |
| PE Dazzle | | | | | |
| PE Vio770 | IFNγ | - | - | 0.6 | 3.6 |
| Alexa 647 (APC) | TNFα | - | - | 2 | 12 |
| APC Fire750 | CD45RO | 2 | 12 | | |
| **20.5 µl/rxn;** | PBS | 12.3 | 73.8 | 17.5 | 105 |

| | Ex vivo Gd-p1 | Surface µL | x 3 |
|---|---|---|---|
| V450 BV421 | PD1 | 2 | 6 |
| V525 BV510 | Aqua L/D | 1:500 | |
| V670 BV650 | CD16 | 1.5 | 4.5 |
| B530 FITC | Vδ2 | 1.2 | 3.6 |
| B710 PerCPeF710 | CD25 | 2 | 6 |
| Y590 PE | CD28 | 2 | 6 |
| Y615 PE Dazzle | CD27 | 1.5 | 4.5 |
| Y780 PE Vio770 | CD3 | 0.5 | 1.5 |
| R670 APC | Vδ1 | 1 | 3 |
| R780 APCFire750 | CD45RA | 1.8 | 5.4 |
| **20.5 µl/rxn;** | PBS | 7 | 21 |

| | Ex vivo Gd-p2 | Surface µL | x 3 | Intra µL | x 3 |
|---|---|---|---|---|---|
| BV421 | Vδ2 | 1 | 3 | | |
| BV510 | CD3 | 1.5 | 4.5 | | |
| BV650 | NKG2D | 2 | 6 | | |
| | Streptavidin | {1.5} | | | |
| Alexa 488 (FITC) | dg9 | - | | 3 | 9 |
| PerCPVio700 | CD56 | 1 | 3 | | |
| PE | PD1 | 1.5 | 4.5 | | |
| PE Dazzle | | | | | |
| PE Vio770 | NKG2A | 0.6 | 1.8 | | |
| APC | Vδ1 | 1 | 3 | | |
| APC Fire750 | Horizon L/D | 1:1000 | | | |
| **20.5 µl/rxn;** | PBS | 11.9 | 35.7 | 17.5 | 52.5 |

Infant thaw @ 12:14 pm
12:54 → stain
1.38 pmPMA ii
L/D @ 5:44 pm
1st FBS spin 6:04 pm
Tetramers @ 6:30 pm (ND006 non-prevax ~0.5µl)
Surface @ 8:36
Sals @ 6:45
RBC lys 8:58-59
Last FBS spin 7:04
Strep @ 7:22 pm
Intra surface stain @ 8:36

12 × 1.8 = 9.6 → 10 mls 20µl L/D Aqua
20 × .5 = 10.0 mls
3 mls → 3µl L/D Horizon
1-9 mls RPC lyse
12 + 11 = 23 − 8 = 19 → 38µl
3 + 6 = 9µl × 9 ml
1M · 2.84 → 2.84
1.5 · 3.52 → 5.28
4 · 2.45 → 9.8
4 · 3.06 → 12.06
2 · 1.58 →
12.64 − 4.5 = 8
17.34 − 4.5 = (5M) 12.8
4 × 1.5 = 2
4 2
3.8 → RNA
3.9 × 1.5 = 13.5
14 mls 14.0 − 1.4 = 12.6

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HEU: Ex-vivo γδ CBMC panels #2

| Specimen | Status | Location | Conc | Date | Tasks | Volume | Ly | Ly+Mon | Total | 1E+6 | 1.5E+6 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Inf 055 a-2 | | RVQ5 | | | | 4 | 2.45 | 3.88E6 | 9.8 | | 612µl |
| Inf 374 a-2 | HEU-B | SV4T | | | | 4 | 3.06 | 4.24E6 | 12.24 | | 490µl |
| Inf313-mo a-2 | HEU-H | SNFD | | 10/1/21 | | 2.5ml | 1.45 | 2.74E6 | 3.625 | 6.85 | 1:pretend 3rd cell |
| | | | | | | | | | | | |
| | | | | | | | | | | | |

730µl 610µl 560µl

443

NKT: 0 0 / 0 0 MAIT: 0 / 0

APC Tetramer

| | | | | |
|---|---|---|---|---|
| BV421 | CCR6 | 2.0 | 9 | 13 |
| BUS10 ~~L/D Aq~~ | L/D | | | |
| BV650 | | | | |
| Fitc | CD4 | 1.8 | 8.1 | 11.7 |
| PerCp | CD45RA | 2 | 9 | 13 |
| PE | CD161 | 0.5 | 2.25 | 3.25 |
| PEDazzle | CD27 ~~CCR7~~ | 1.5 | 6.75 | 9.75 |
| PEVio770 | CD3 | 0.5 | 2.25 | 3.25 |
| APC | Tetramer or Va | | * | |
| APC Fire750 | CD8 | 1.5 | 6.75 ~~4.25~~ | 9.75 |
| PBS | PBS | ~~5.2~~ | 41.4 | 59.8 |

Accidentally became CD27

10.7 - tetramer 9.2/5.7 APC 4.5 tetramer

Va5

| | | | | |
|---|---|---|---|---|
| BV421 | Va24 | 2 | 4 | 6 |
| BUS10 | CD3 | 1.5 | 3 | 4.5 |
| BV650 | NKG2D | 2 (1.5) | 4 | 6 |
| Fitc | ~~CD4~~ | ~~1.8~~ | ~~3~~ | CD4 → 3.6 |
| PerCp | CD56 | 1 | 2 | 3 |
| PE | PD1 | 1.5 | 3 | 4.5 |
| PEDazzle | CCR7 | 1.5 | 3 | 4.5 |
| PeVio | NKG2A | 0.6 | 1.2 | 1.8 |
| APC | CCR6 | 2.5 | 5 | 2.5 |
| APC-Fire | L/D | ~~7.4~~ | | — |
| | PBS | 6.4 - 1.5 = | 9.2 | 22.6 |

Va5

| | | | | |
|---|---|---|---|---|
| BV421 | Va24Jα18 | 1.5 | 6.75 | |
| BUS10 | L/D Aqua | | | |
| ~~BV650~~ | CD4 | 1.8 | 8.1 | 5.4 |
| Fitc | CD45RA | 2 | 9 | 6 |
| PerCp | CD161 | 0.5 | 2.25 | 1.5 |
| PE | PEDazzle CD27 ~~CCR7~~ | 1.5 | 6.75 | 4.5 |
| PEVio | CD3 | 0.5 | 2.25 | 1.5 |
| APC | CCR6 | 2.5 | 11.25 | 7.5 |
| APC Fire | CD8 | 1.5 | 6.75 | 4.5 |
| | PBS | 8.7 | 39.15 | 26.1 |

| | | | | |
|---|---|---|---|---|
| BV421 | CCR6 | 2 | 4 | 6 |
| BUS10 | CD3 | 1.5 | 3 | 4.5 |
| BV650 | NKG2D | 2 | 4 | 6 |
| Fitc | ~~CD4~~ | ~~1.8~~ | ~~3~~ 3.6 | CD4 → 5.4 |
| PerCP | CD56 | 1 | 2 | 3 |
| PE | PD1 | 1.5 | 3 | 4.5 |
| PEDazzle | CCR7 | 1.5 | 3 | 4.5 |
| PeVio | NKG2A | 0.6 | 1.2 | 1.8 |
| APC | Tetramer | — | — | — |
| APC-Fire | L/D | — | — | — |
| | PBS | ~~10.4~~ - tetramer | | |

8.6 - 1.5 = ~~14.2~~ 21.3

Cytometer failed to acquire ...

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HEU: Ex-vivo γδ CBMC panels #2

No adjustments to PMT, mixer for BUV650 scgate

PMA L/D @ 7:52pm →

7:55pm → ~~Lots~~ FixPerm for P2/P4b

P2 Va NRT PFAed & stashed

8:20 tetramer 4 sc → 8:23

PMA FBS wash @ 8:08

→ Last tetramers PFaed & away

PMA surface @ 8:20

8:29 → 2nd Perm wash P2/P4b

P2 P4b intrac @ 8:38 → 9:18

PMA RBC lyse @ 8:41 → 8:44 ✓

P2 P4b done @ 9:30pm

PMA int @ 21:45 ✓

Done @ 22:37 pm ✓

3
15
×7
105 → 2hr

start ctl - Inf055 ctrl

FSC is @ 610

Pump is dripping, but humming.
~7 minutes/tube?

Conc/ @200 evts/sec

$\frac{1.5}{.150} = 10E6/ml$

conc drops ~2000,
↑ around 6:45 minutes

(Inf374 lowest conc vs other 2)

12
×7
84

P2 055 → 7000 evts @ min 3!

Finishing P2 → ~12:34

P1 → max Fsc back to 600

10,000 evts @ 4:14 p1 Inf055 hotfired!
(lot of RBCs surviving)

LSR Froze @ 1:12 pm

restart files ✓ @ 1:19 w/o need to restore

P1s just seem to be running @ high conc
@000 evts/sec due to RBCs.

Erica 2 & 6 FSC-610

to ctl middle section, ~2 hr ~~gap.~~ expected run time.

Restarting @ MAIT tet tubes, 6000 evts/sec (RBC's not lysing)

L/D CD3 FSC x axis was on biexponential...

11,000 evts gets tech ~1404 evts abort rates

ND006 has barely any NRTs ....

15,000 evts - 2500 evts aborted/sec.

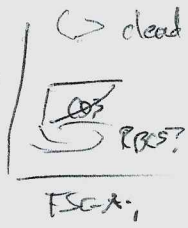
← Cytometer issues

↑ FSC 610 @ Inf374 ... Sigh running on Low !!

3 minutes later realized

10/2/21 5:20:36

LSR acting really bizarrely....




upper

I used 2µl instead of 1.5µl Va24 jal8.... for P2 wells.

¿ discrepancy Va vs tet stainings ?

The Va reg ≠ Va P2, something off