

October 5-6<sup>th</sup>, 2021HEU: Ex-vivo  $\gamma\delta$  CBMC panels #4[illegible]

	Ex vivo Gd-p4b	Surface μL	Intra μL
BV421	PD1	2	2
BV510	Aqua L/D	1:500	1:500
BV650	CD56	1.8	1.8
Alexa 488 (FITC)	Perforin (dG9)	-	-
PerCPeF710	CD3	1.5	1.5
PE	GZMb	-	-
PE Dazzle			
PE Vio770	NKG2A	0.6	0.6
APC	V82	1	1
APC Fire750	CD16	1	1
20.5 μl/rxn;	PBS	12.6	12.6

	Ex vivo Gd-CK PMA	Surface $\mu\text{L}$	8 x 6	Intra $\mu\text{L}$	8 x 6
BV421	PD1	2	12 16		
BV510	Aqua L/D	1:500			
BV650	CD27	1.5	9 12		
FITC	V82	1.2	7.2 9.6	0.2	1.2 1.6
PerCPeF710	CD3	1	6 8	0.2	1.2 1.6
PE	CD56	0.5	3 4		
PE Dazzle					
PE Vio770	IFN $\gamma$	-	-	0.6	3.6 4.8
Alexa 647 (APC)	TNF $\alpha$	-	-	2	12 16
APC Fire750	CD45RO	2	12 16		
20.5 $\mu\text{L}/\text{rxn}$ ;	PBS	12.3	73.8	17.5	105 140

	Ex vivo Gd-p1	Surface μL x 34
V450 BV421	PD1	2 68
V525 BV510	Aqua L/D	1:500
V670 BV650	CD16	1.5 4.56
B530 FITC	V82	1.2 3.648
B710	CD25	2 68
PerCPeF710	CD28	2 68
Y590 PE	CD27	1.5 4.56
Y615 PE Dazzle	CD3	0.5 1.52
Y780 PE Vio770	V81	1 34
R670 APC	CD45RA	1.8 5.472
R780 APCFire750	PBS	7 2128
20.5 μl/rxn;		

	Ex vivo Gd-p2	Surface μL x 3	Intra μL x 3
BV421	V82	1	3
BV510	CD3	1.5	4.5
BV650	NKG2D	2	6
	Streptavidin	{1.5}	
Alexa 488 (FITC)	dg9	-	3
PerCPVio700	CD56	1	3
PE	PD1	1.5	4.5
PE Dazzle			
PE Vio770	NKG2A	0.6	1.8
APC	V81	1	3
APC Fire750	Horizon L/D	1:1000	
20.5 μl/rxn;	PBS	11.9	35.7

$\frac{70}{16} \text{ Agun}$   
 $\frac{16}{x.8}$   
 $12.8$   
 $20 \times 3 = 60$   
 $\pm 10$   
 $70 \text{ ml } \text{FB5-PB3}$   
 $\frac{1.0}{4} \text{ pt - 9 bowl}$   
 $\frac{22}{4.5}$   
 $\frac{110}{10}$   
 $\frac{5}{12} \text{ Perm wash}$   
 $\frac{x.3}{64}$   
 $\frac{14.6}{28.1}$   
 $\text{P4b P2}$   
 $\text{don}$   
 $\text{CL-Suspension}$   
 $98.23$   
 $\text{Intra}$   
 $@ 9:07$   
 $\text{Panz @ 8:01}$

Pub/P2 arena <sup>step 5</sup> 4706  
 done @ 6:03 pm 6-19  
 Suprem 18:23  
 arena @ 9:07  
 28:01  
 Pub/P2 intra @ 5:02 pm 5:42  
 PMA sign @ 5:04 pm  
 arena RBC 5:12 → 5:15  
 PMA 40 @ 5:17 → 5:32  
 44-Swivel/P2 5:50  
 Pth/P2/P1 RBC sign @ 3:31  
 P15 done @ 3:47  
 step @ 3:53 pm → 4:08  
 arena 5(12) done @ 3:55  
 arena 38° @ 4:00 → 4:30  
 Fox Pen @ 10:21 55  
 arena 42° @ 4:40 → 5:00

9:10 stain count  
PMA: 11:01 ✓  
11:21 AM spendown  
~~08:21 pm start~~  
h/d @ 2:18 pm  
2:32 sds in  
R Tote @ 2:36  
+40 → 2:36  
Fork pipette  
screw p.  
Inf 27 got  
2 PD.  
/ In 29 PI  
was delayed  
5:05 → 25  
(5:13 → 20)  
2) sds for pms  
3:16  
reg sds done @  
3:20 pm  
1/2 ml FBS - FBS



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Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	1E+6	1.5E+6
Inf 21-2 a-2	RR9P HU					2	3.46	4.10 EG	6.92 M		700 <sup>HH</sup> 285 <sup>HH</sup>
Inf 27-9 a-2	RR5T HEU-L					2	1.70	2.63 EG	3.4 M		1 M $\rightarrow$ the box
Inf 170-8 a-2	S92T HEU-H				ANTS! This is how we get ants!	2	1.22 <del>2.59</del>	1.82 EG	2.4 M		
Inf 197-7 a-1	SAM6 HEU-H					2	2.59	3.64 EG	7.23 M		
Inf 197-7 a-2	SAM7 HEU-H					2	2.31	3.32 EG	6.64 M		730 <sup>HH</sup> (95+175) 210

Inf 29  $\rightarrow$  15.2 M  
 $\rightarrow$  Inf 27-9  $\rightarrow$  7.52  $\sim$  6 M  
 Inf 170  $\rightarrow$  5.72  
 Inf 197: 13.92  
 ND050: 7.82 M  
 6.10  
 5.126  
 2.59  $\sim$  11.2  
 1.16 EG / tube  
 4.12 EG  
 5.28

LSR-II run wednesday upped p<sub>e</sub> 635  $\rightarrow$  640<sup>HH</sup> p<sub>e</sub> Dazle 840  $\rightarrow$  645 10:20 AM ready @ 10:28 AM

IVL FSC  $\rightarrow$  615  $\sim$  4 min (@ 100  $\mu$ l) 2 drops remaining volume @ spike (by dropper)  $\sim$  4:30

Inf 197 p4b  $\neq$  CD3? makes no sense, given cells  $\neq$  No fhy cl.

both INF 29 + 170 worked and CD3 worked in the other-panel w same color

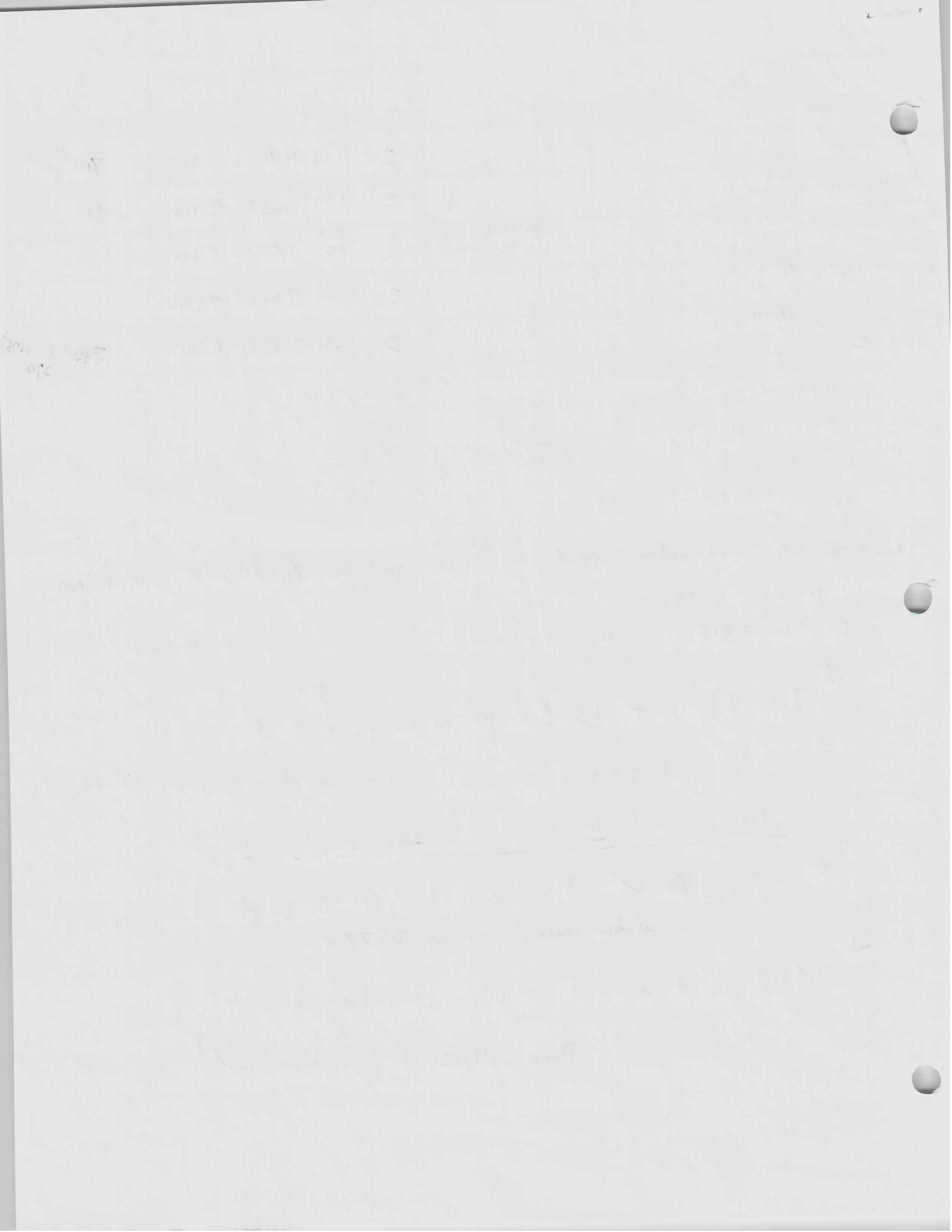
Inf 197 p1 fewer than 1M cells in its tube - per tube note.

P<sub>i</sub>  $\rightarrow$  FSC  $\checkmark$  605  $\checkmark$  P<sub>i</sub> comp is crazy off CD3 - pop - Inf 29 - window scale? not on Inf 27  $\checkmark$

Inf 197 P<sub>i</sub>  $\neq$  expected  $\neq$  VD2 ... not enough events?

Done @ 12:15 PM (2 1/2 hr window  $\checkmark$ )





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	PMA wells		<u>A<sub>1</sub></u>	<u>A<sub>2</sub></u>
Inf 29	1.5	4.15	361 $\mu$ l	3.41 439 $\mu$ l
Inf 27	1	2.00	485 $\mu$ l	1.70 666 $\mu$ l
Inf 176	1	1.64	603 $\mu$ l	1.83 819 $\mu$ l
Inf 197	1.0	2.59	579 $\mu$ l	2.39 <del>427</del> $\mu$ l

560  $\mu$ l

700 - 301 : 339

700 - 485 : 215

700 - 603 : 998

700 - 427 : 273

Q2:  $\rightarrow$  split 3 ways Inf 197 1M P<sub>4b</sub>, P<sub>2</sub>, ~ P<sub>1</sub>  
 Inf 29 all 3 gd 1.5  $\checkmark$  ~ NET

1-1-1

1-1-1

1-1-1

1-1-1

1-1-1

1-1-1

1-1-1