

November 17th, 2022

Monocytes #6
Tetramer Titrations and Monocytes

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	2E+6
ND006	500pl test		7.5 E6	11-14-22	clumpy cells	1	3.11	4.20	3.11	0.964	
ND006	1ml		10E6	11-14-22	sat longer @ 80°C	2	4.14	5.46	8.28	0.724	
ND006	1ml		10E6	11-14-22	yesterday?	2	5.23	6.50	10.46	0.573	
ND006	1ml		10E6	11-14-22		2	4.83	6.17	9.66	0.621	
									31.51		

check 500pl for viability subsequently
2:16pm stain count

Ab's prepped @ 4:16pm

No ~~Ab~~ stim condition
thus go around

4:53 pm → cell spin

5:01 L/D → 22

5:13 1st sc's → 43

spin @ 5:26pm

add sc's @ 5:36pm → 6:06pm

37°C samples @ 5:44, 37°C sc's out. → 6:14pm

5:48 RBC lysis → 5/52

I forgot Fe Block w/ LD

06:00 PM 37°C + 3pl Fe block (15 minutes later!)
re-added to the incubator → 6:20pm

4°C sc's 06:00-06:08 RBC lysis → 6:10pm
[37°C x PFA add → fix] spin @ 6:13pm

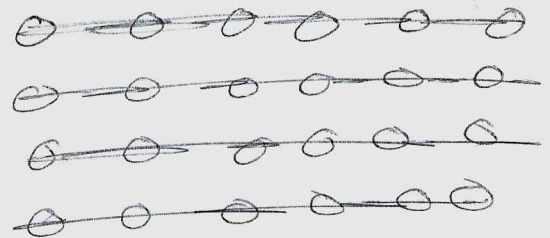
samples spin @ 6:24pm

sc's done @ 6:29pm

6:41 sc's 4°C in → 7:11pm

Done @ 7:38pm (bichazard & coverage tips also to cubedane)

CD64 CD123 CD66 FcRn
+ sc's + unstained
old New
scyl



$$\begin{aligned}
 &27 \text{ sc's} \\
 &23 + 3 = 26 \text{ sc's} \\
 &\quad \quad \quad \text{e.3} \\
 &\quad \quad \quad 7.8 \text{ M} \\
 &\quad \quad \quad 21.0 \text{ M} \\
 &\quad \quad \quad \hline
 &\quad \quad \quad 28.8 \text{ Million Cells}
 \end{aligned}$$

$$\begin{array}{r}
 8 + 27 \\
 \times 8 \quad \times 2 \\
 \hline
 64 \quad 54 \\
 \hline
 \end{array}$$

$$3 * 50 \times 4 = 200 \text{ f EDTA}$$

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#	Filter	Single color (ul)	Ref ctri type	Unmixing ctri name	Fluorochrome	Marker	Clone	Vial Lot #	L/D + Fc blocker 3ul/1xm	37oC (RT) for 30min	8	4oC for 30min	8
1	UV2	0.6	Cells		BUV395	CD18	(6.7)			0.6			
UV6					AF	AF-UV6							
UV7					BUV496								
2	UV9	0.6	Cells		BUV563	CCR2	(IS132.109)						
UV10					BUV612								
3	UV11	1	Cells		BUV661	CD3GR1	(2A9-1)						
4	UV4	1.2	Cells		BUV727	CD19				1		8	
5	UV16	1.0	Cells		BUV805	CD14	(M5E2)					1.2	9.6
V1		1.0	Cells		BUV421	FCER1a						1.0	8
V3		1.0	Cells		Pacific Blue	CD66b						<1>	
V5		1.2	Cells		BUV480							<1.2>	#VALUE!
20	V7	1.2	Cells		BUV510	CD64						<1.2>	#VALUE!
6	V8	1.5	Cells		BUV570	CD16	(3G8)					1.5	12
7	V10	1.0	Cells		BUV605	CD56	(5.1H11)					1.0	8
8	V11	1.0	Cells		BUV650	CD11c	(B-ly6)					1.0	8
21	V13	1.2	Cells		BUV711	FCER1a						<1.2>	#VALUE!
V14		1.2	Cells		BUV750								
9	V15	1.2	Cells		BUV785	CD141						1.2	9.6
10	B2	0.4	Cells		FLITC	CD11b						0.4	3.2
B3					Spark blue 550								
11	B4	0.4	Cells		PE	CD88	(SS/1)					0.4	3.2
12	B6	0.6	Cells		PE-Dazzle594	CD3						0.6	4.8
13	B8	0.5	Cells		PE-Cy5	CD86	(IT2.2)						
B10		0.5	Cells		PerCP-Cy5.5	CD123				0.5	4		
14	B13	0.4	Cells		PE-Vio770	HLA-DR	(REA805)			<2>		0.3	2.4
R1					APC								
15	R2	1.0	Cells		Alexa Fluor 647	CD163	(GH/61)					1.0	8
16	R4	1.0	Cells		APC-R700	CD40	(5C3)						
17	R6	3001	Cells		Zombie NIR	L/D			<1.2500>				
22	R7	1.2	Cells		APC/Fire 750	CD123	CD64					<1.2>	#VALUE!
18	R8	1.0	Cells		APC/Fire 810	CD38	(HIT2)			1.0	8		
+ SC unstained					True Stain FcX					0.5	4	0.5	4
Antibody Total													
Brilliant Stain													
Pipette draw volume/sample													
And UNSTAINED CONTROLS !!!													
										4.6	36.8	10.7	85.6
										50	400	50	400
										53.6		59.7	

Simplified Protocol

Aliquot cells 3E+6 cells/tube

Wash with 2ml PBS, 1300rpm, 8min

2500x diluted Zombie NIR, 1E+6/1ml + 3ul Fc blocker at RT for 15min

Wash with 2ml 2%FBS-PBS 2min EDTA

Spin at 1300rpm for 8min

x ul of 37oC Ab mix, at 37oC for 30min

Remove RBC with 1ml lysing solution for 3min

Wash with 2ml 2%FBS-PBS 2min EDTA

Spin at 1400rpm for 6min

x ul of 4oC Ab mix, at 4oC for 30min

Treat with 600ul of 1x lysing solution at RT for 3min

Wash with 2ml 2%FBS-PBS 2min EDTA

Spin at 1400rpm for 6min

Resuspend in 0.4%PFA-PBS

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[illegible]

~~extra sets~~



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PaenAP unmixed @ 12:50pm

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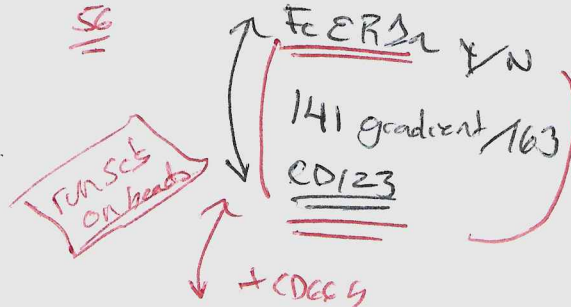
10:30 se acquisition

1200 cells/sec for 50,000 40s

CD123 @ 200,000 events

FEER1A \neq

SG



// is CD66b suffering from AF effect?

// Ab amount by volume or?
by cell? both //

Done @ 12:30 pm

Including doublet AF uniqueness
for their characterization

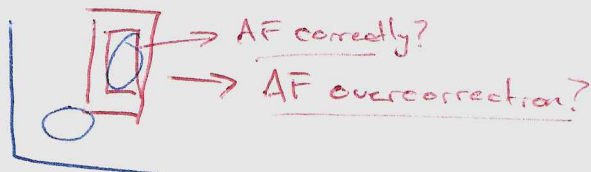
Unmixing:

CD40 needs unstained swapped to lymph

after 1st
unmixing
issues

Swapped out APC-Fic750 for CD66b

PerCP-Cy5.5 123 \rightarrow TNF α PBMC α (library)



3 signatures in monocytes

green & red overlap in V, not W;
(231) (13311)
orange (over both)
(2211)

CD66b unmixed \neq (very dim marker)

(correct LxDead \rightarrow viability)

(Lost screenshots (didn't save)
but swapping to library controls
 \uparrow 5 the panel)

Cells 66b+ result not
Fc Blocking correct
- CD66b
CD3CR1

some CD8
some CD19
some CD4
some CD56
some CD40

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123 suffers more spreading on APC Fc750 ^{negative} (subjected to spreading error)

123+ \uparrow in-vitro (4%) for ~~the~~ CD11c

86 \uparrow

but CD123 is brighter more stretched

Pdcs ~~low~~ low \downarrow

\approx 16 was brighter mold?

CD123 on APC Fc750 > PerCP-Cy5.5 (despite neg spread)

w/ caveat spread upon activation

FCER1a \downarrow

improved (and spared AF management)

CD64 marginally improved

\rightarrow \sim equivalent but now at mercy AF management

3 AFs for Monocytes:

Main \sim Violet, \downarrow UVs.

Side \sim \uparrow UVs but not Vs.

dead/dying? \downarrow both.

$V_3 \times UV_3$

$UV_5 \times V_7$

$UV_3 \times V_{10}$

- APC - Fc750 CD64
- ✓ Htt - Sur(4) CD123 PerCP-Cy5.5
- ✓ DTR PBMC6 - CD19-202c Pac Blue

New Old

One af	0 w/o	0 w/	0 w/
0 Yes af	0	0	0

My tags are not "pure" per se....

No AF UV3 tag

88 \times 64

"doesn't look like this is the tag we are looking for."

So FCER1a on BV711 \checkmark
CD123 PerCP-Cy5.5 better \checkmark see what happens in combination
CD64 on

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