

November 29th, 2022

Monocyte #8

1:100

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	2E+6	.3E+6
NY004							~1.5	~3.2			

LD @ 2:52pm → 3:07pm
w/ FCBlood @ 4pxl

Spine @ 3:09pm → 3:

hot 3:20 → 3:50pm

mixes done @ 3:31pm

Cold @ 4:06 w/ spiked → 4:37pm
cells

(cells clumping tubes will filter
@ 300 p1 RBC step)

RBC lyse @ 4:39pm

Silvered & spinning @ 4:45pm

Done @ 4:57

UV1 UV2 UV3

Avem Machine on @ 10:28am →

#	Filter	Single color (ul)	Ref ctrl type	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial lot #	L/D + FC blocker 3ul/rxn	37°C (RT) for 30min	2	4°C for 30min	2
1	UV2	1.25	cells		BUV395	CD18	(6.7)			0.6	1.2		
UV6					AF	AF							
UV7					BUV496								
2	UV9	1.25	cells		BUV563	CCR2	(1s132.1D9)					0.6	1.2
UV10					BUV661	CD19	(2A9-1)						
3	UV11	1.25	cells		BUV737	CD19	(M5E2)			1	2		
4	UV14	1.25	cells		BUV805	CD14	(M5E2)					1.2	2.4
5	UV16	1.25	cells		BUV421	CD80						1.0	2
6	V3	1.25	cells		Pacific Blue	CD66b				<1.2>	2.4	<1.2>	#VALUE!
7	V7	1.25	cells		BUV480					1.2	2.4		
8	V8	1.25	cells		BUV510	CD64						1.2	2.4
9	V10	1.25	cells		BUV570	CD16	(3G8)					1.5	3
10	V11	1.25	cells		BUV605	CD56	(5.1H11)					1.0	2
11	V13	1.25	cells		BUV650	CD11c	(B-ly6)					1.0	2
V14					BUV711	FCER1a						1.2	2.4
12	V15	1.25	cells		BUV750								
13	B2	1.25	cells		BUV785	CD141						1.2	2.4
22	B3	1.25	cells		Spark Blue 550	CD11b						0.4	0.8
14	B4	1.25	cells		PE	CD88	(SS/1)					<1.2>	#VALUE!
23	B6	1.25	cells		PE-Dazzle594	CD3						0.4	0.8
15	B8	1.25	cells		PE-Cy5	CD86	(IT2.2)			0.5	1		
24	B10	1.25	cells		PerCP-Cy5.5	CD123				2	4		
16	B13	1.25	cells		PE-Vio770	H1A-DR	(REA805)					0.3	0.6
R1					APC								
17	R2	1.25	cells		Alexa Fluor 647	CD163	(GH/61)						
18	R4	1.25	cells		APC-R700	CD40	(SC3)			1.0	2	1.0	2
19	R6	1.25	cells		Zombie NIR	L/D							
25	R7	1.25	cells		APC/Fire 750					<1.2500>			
20	R8	1.25	cells		APC/Fire 810	CD38	(HIT2)			1.0	2	<>	#VALUE!
					True Stain FcX					0.5	1	0.5	1
Antibody Total										6.6	13.2	13.7	27.4
Brilliant Stain										50	100	50	100
Pipette draw volume/sample										55.6		62.7	
And UNSTAINED CONTROLS !!!													

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-2mM EDTA
Spin at 1300rpm for 8min

x ul of 37°C Ab mix, at 37°C for 30min
Remove RBC with 1ml lysing solution for 3min

Wash with 2ml 2%FBS-PBS-2mM EDTA
Spin at 1400rpm for 6min
x ul of 4°C Ab mix, at 4°C for 30min
Treat with 600ul of 1x lysing solution at RT for 3min

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

Spin at 1400rpm for 6min
Resuspend in 0.4%PFA-PBS

Spiked in indiv

51.6

59.7

210

210

210

11/29/2022

[illegible]

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Spark 550 unmixing w/o any AF extract (on CD3 gate)

~~CD66b~~, ~~CD66b~~ swapping manual 0 sec's

CD66b - CD3

CD2 tail debris (v. minor)

CD19

14,

(66b major errors)

64

11c stream

CD141 struggling ↓

88 tail stream

163 tail

major CD3 off,

Spark 550

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