

November 22nd, 2022

(reusing last weeks ref controls)

Don't forget Fe block!!

Monocytes #7

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	2E+6	2E+6
NDCOG ₍₁₎	Adult		15E6	11-14-12						3E+6	2E+6
(2)											316f1
(3)											389f1
(4)											366f1
(5)											425f1
											448f1

6 ml 2% FBS-PBS(+EDTA) * 9 = 54

2 ml + 12 Sc's = 24
78 ~ 100nls

50
x 4
200f1 EDTA
10 + 40 mls PBS
FBS

21
+ 9
30
x 1
3.0

8x800 = 6.4 mls Zombie NIR

15x300 = 4.5 mls RBC lyse 1x

3:47 pm w/d stain → 4:02 pm

3:57 pm Sc's @ 4°C (in CD80) → 4:27 pm ✓

✱ Forgot the 3f1 of blood, will add in @ separate 15 min period while tetramers stain

remedial @ 4:19 pm → 4:34 pm

Tetramers @ 4:27 → 5:07 pm

Rbc lyse cell sc's @ 4:29 pm → 4:32 pm

lysed Sc's + Fe Blockd monocytes spinned @ 4:36 pm ✱

4:55 37°C for monocytes in ✓ → 5:25 pm

✱ Tetramer spin @ 5:11 pm

37°C monocytes at @ 5:27 pm, spin @ 5:31

4°C abs indiv + mix added @ 5:54 pm → 6:24 pm

6:28 → 6:51 pm

Mon 4°C spin @ 6:34 pm

Done @ 6:48 pm

○ unstained for samples

⊗ Backbone (BV711 FeR1a + BV510 CD64 + Pac Blue CD66b + R1Dazle CD3)

⊗ Backbone + R1Dazle + BV421 CD3

⊗ extra + R1Dazle + BV421 CD80

⊗ Backbone + R1Dazle CD3 + APC-Fire750 CD123

⊗ Backbone + R1Dazle + CD123 CD3

⊗ Backbone + R1Dazle + APC-Fire750 CD3

⊗ Backbone + BV421 CD3

⊗ Backbone + Spark Blue CD3

⊗ Backbone + APC-Fire750 CD3

+ Bead controls

Const ⊗ CD66b ⊗ CD123 ⊗ CD123 ⊗ FeR1a ⊗ FeR1a
PacBlue APC-Fire750 BV421 BV711

+ Sc controls (7x3) = 2.1 ml

⊗ CD3 ⊗ CD3 ⊗ CD3 ⊗ CD3 ⊗ CD4
PacBlue BV421 Spark Blue APC-Fire750 APC-Fire750

Ultracomp Beads ~ 1 drop

all got 1.2 fl ~ 1 ab test volume

30 min @ 4°C

Wash 2-3 mls FCS buffer

400-600g 3-5 min

resuspend in 0.4% FCS-PBS

Beads @ 7:44 4°C → 36

62.1	586	10/11
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Aliquot cells 3E+6 cells/tube

2500x diluted zombie NIK, 1E+6/1ml + 3ul Fc blocker at RT,

Spin at 1300rpm for 8min

Remove RBC with 1ml lysing solution for 3min

Spin at 1400rpm for 6min

11/22/2022

[illegible]

CD3 CD3
Dazzle Spark Blue
+ vest panel

Paragys CD123

BV750/AF647 CD4



November 22nd, 2022

10:07 AM

Monocytes #7

Wednesday 11/23/22 @ 9:37:00: Aurora off (day before Thanksgiving),
tube of antibody? $\frac{1}{2}$ & $\frac{1}{2}$ waste/sheath tank. Jessica Allen last user

Blue FSC laser has been failing (ask how to do QC on Aurora)

2004 lot beads (in the drawer of the large
fridge in 7-0224)

360 μ l a drop

300 μ l

$\square \downarrow$ real life + 1 drop Lot 2004
QC beads.

Again to match MFI % CV spread peaks

10:06 QC on 2004's // Passed \checkmark

- Duplicating monocytes taking its time

25,000 beads

Pretty sure CD80 \neq worth on cells

APC-Fc γ 750 looks dim? vs other channels (ditto for CD44, yeah!)

\checkmark ← Beads on monocyte schedule

Acquire on low? (didn't run unstained
beads (yet!))

Sample acquisition @ 11:03 pm

\uparrow 2000 events/s

Aurora 3 Laser is down

scheduled myself T/R 9:30-12:00 for

Monocytes done @ 12:00 pm


next 5 weeks on SL

Unmixing ~ Nov 23rd, 2022 °

1st attempt CD80 BV421 beads ~~CD3~~
PacBlue CD666 ✓
BV 711 FcER1a ✓
PerCP-Cy5.5 ID123 ✓
APC-Fc750 CD123 ~~CD4~~ ~~CD3~~

Internal bead controls
* same FSC/SSC worked

* Are brightest just bright, or are they junk/doublets (k_2 AF) etc.
is it poisson noise or something else

What weight the  has?

Bright or brightest is a lie *

* CD38* brighter on Lymphocytes / CD40 (swap the negs)

* does my general AF gate impact?

Center it on monocytes vs all ^{myeloid} CD88 ~ linked AF production

CD88, HLA-DR, CD11c other 2 notable signders: Leaving as is for now
(no tag added);

Your gated events are ~ all cell based

→ 99.5%
are singlets once gate
on a pop using FSC-A/
SSC-A

w/o Tag → FcER1a blankish (CD80 stain is pulled up in B cells)

CD3 BV421 vs PacBlue / CD64

dazle 594 11c/

Very few issues on Spark Blue 550

PerCP-Cy5.5 > APC-Fc750
CD123

* CD4 decently behaved
on APC-Fc750

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Monocytes #7

New unmixing

AF tags vs Extract + UV6 tag
w/ or w/o gating brightest vs centered peaks

* HLA-DR CD88 ~~FeR1~~ CD11c getting band treatment

BV421 CD80 beads }
666 beads

FeR1a beads }
123 Beads

APC Fe250 CD4

CD3 dazzle 123 G5 80BV421

≥ * Ran a sc unstained * ? on PacMap
→ check my "titration log"

Tag in No AF ← CD64 for ^{True} monocyte gate
less CD8 spill ↓ on cDC1 gate

FeR1a (beads)

Yes tag in Yes AF ←

≥ BV421 on ? no...

Myeloid PacMap → no CD3, CD19, Zombie
or AF/UV6's

277,179 @ 10:31 pm

1.5

700

500

280

800,000 T cells

200,000

220,000 P

November 22nd^h, 2022

Monocytes #7

IFN γ	(6)	5
CCR4	(6)	5
CCR2	(2)	2
CXCR4	(1)	-
BV0237	(2)	1
CCR5	(1)	1
AFM1	(1)	1

OS
u
02:11

5/100/5

CD62L	(4)	4
BV0496	(2)	1
BV0563	(6)	5
CCR4	5	(4)
Ud2	(5)	4
CXCR3	5	(5)
CD4	(4)	3
161	(2)	2
CD7	(1)	-

