

February 1st, 2023

PMA: FBS Lots

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	.3E+6
NY062						2	5.46	8.30	10.92 16.60		
ND006						2	6.34	8.04	12.68 16.08		

10:50 AM (post locator delivery)

Thawed ND006 → Bioassay

NY062 → water bath

note: blurb L2 bubble, slight unsealed lid?

Into FBS Atlantic RIO (01/13/2023 sterile)

for spin.

Resuspend in UO1 PBS (2mls)

but then spin down? resuspend in clean bath.

(11:02 AM stain)

One used last time

	Atlantic	UO1
	PMA ctrl	PMA ctrl
NY062	0 0	0 0
ND006	0 0	0 0
	unsealed	
	0 +1	
	0	
	0 +1	

+ Full sets of SC's

11:39 AM AM aliquots spinning down in tubes

1200 rpm/10 min

or extra ml UO1 PBS for wash (capped? ups)

(11:58 AM Aliquotted)

466 µl → 534 µl RIO + 2 µl

233 µl + 267 µl RIO + 1 µl SC's

315 µl + 685 µl RIO + 2 µl

157 µl + 343 µl RIO + 1 µl

NY062

ND006

NY062 4M

ND006 4M

5M

5M

$$\begin{aligned}
 & 2000 \\
 & 4\% \cdot 5.46 = 0.732 \\
 & 40 + (366 \mu l) \leftarrow 1.268 \\
 & 4\% \cdot 6.34 = 0.631 \\
 & \leftarrow 1.369 \\
 & \boxed{315 \mu l} \leftarrow
 \end{aligned}$$

4M 2M/tube as condition

$$\begin{array}{r}
 10.92 \\
 - 9 \\
 \hline
 1.92
 \end{array}$$

$$\begin{array}{r}
 12.68 \\
 - 9.00 \\
 \hline
 3.68
 \end{array}$$

ND006 as SC's

Incubator @

12:34 pm

(w/ CD107a 6 µl)

1 ml PMA Atlantic (4)

1 ml Ltl Atlantic (4)

250 µl PMA ctrl (2)

1 ml PMA UO1 (4)

2 ml ctrl UO1 (8)

$$\frac{250 \mu l}{500 \mu l} = \frac{PMA}{2}$$

Aurora bouncing

37°C mix made @ 5:34pm
ABU480 done, no CO2,
new CO23 bottle,

Abs prepped @ 5:46pm

Reagents prepped @ 6:20am

@ 6:34

Add Zombie to the get
more next time!!
- 30/75 residual
for ANT ND006
distained

SC's aliquoted 50/100 drl
25pl PBS
@ 6:47pm 100pl CO107a

Spin @ 6:53pm

L/D @ 7:16pm → 7:31pm

Hot sc's @ 7:29pm → 7:59pm

Cold sc's @ 7:45pm → 8:15pm

RBC lysis @ 8:01pm 1400 rpm 6min @ 20°C

RBC lysis @ 8:21pm
(cold prep)

65pl 37°C @ 7:51pm → 8:21pm

Spin @ 8:26pm

Tetramers + cold sc @ 8:31pm → 9:14pm

Sc Fix Perm @ 8:44 → 9:04

SC's 1st perm wash @ 9:10pm

2nd perm wash

+

tetramer FBS wash

@ 9:26pm 1400 rpm 6min

AF 488 @ 9:36 → 46 → 56 9:32pm → 1st perm wash @ 9:58pm

SA. 7 4°C @ 9:40 → 10:10
15h 15h ~ 30 ~ 45

Build sc's @ 9:49pm done

SC's intracellular @ 9:55pm → 10:35pm

introduced
mg H2S1

(140-15000 w)

10:12pm RBC lysis samples

10:23pm → filling locator

Samples, 10:27pm → 37 → 47pm

SC's done @ 10:49pm.

1001K-175K-20011

February 1st, 2023 PMA Responses by FBS Lot

#	Filter	Single color (ul)	Ref ctrl	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	During Stimuli	8	L/D 15 min (RT)	Tetramer 40 min @ RT	Host stain 30min @ 37C	8	Cold stain 30min @ 4C	8	RBC Lys, then Fluorim	Spiked 30 min @ RT	8
1	UV2				BUV385	CD82	(DMEG-50)	25%							1.2	9.6			
2	UV7				AP	APC-UV6													
3	UV9				BUV495	CD8	(RPA-18)	10%							0.7	5.6			
4	UV10				BUV568	CD69	(TN50)								0.5	4.0		1	8.0
5	UV11				BUV615	CD84	(1G2)						2.0	16					
6	UV4				BUV641	V2	(166)								0.7	5.6		0.5	4.0
7	UV16				BUV737	CD38	(1C6)								1.3	10.4			
8	V1				BUV805	CD4	(SK3)	33%					1.5	12				0.5	4.0
9	V3				BUV821	CD127	(4D355)	33%					1.5	12					
10	V5				Pacific Blue	CD14	(W5E2)						1.5	12					
11	V7				Pacific Blue	CD19	(H1819)						2.0	16.0					
12	V10				Pacific Blue	CD45	(RE3A31)	12%					2	16					
13	V13				BUV605	CD36		133%							0.7	5.6			
14	V14				BUV650	CD7							1.0	8					
15	V15				BUV711	CD7		64%					1	8				1	8.0
16	B2				BUV750	IFN γ	(B27)	133%					1	8					
17	B3				BUV786	CD36	(11A9)	17%					1.5	12				1.5	12.0
18	B4				Allophycocyanin 488	CD3	(SK7)	83%											
19	B6				PE	CD3													
20	B8				PE-CF594	CD26	(M-A261)	60%					1.5	12				0.5	4.0
21	B10				PE-CF5	CD25	(M-A251)	30%					1.2	9.6				0.5	4.0
22	B13				PE-CF5.5	CD45	(M-A451)											2.5	20.0
23	R1				PE-CF5.5	CD45	(M-A451)											0.5	4.0
24	R2				APC	CD16													
25	R4				APC-CF594	CD107a	(H4A3)	20%											
26	R7				Zombie NIR	CD27													
27	R8				APC-Fire 750	CD27	(0323)	55%											
And UNSTAINED CONTROLS (II)																			
Antibody Total									6.0	48			19.0	152	13.7	109.6		9.5	76
R10 Media									14.5	116			50	400	50.0	400		50	400
Pipette draw volume /sample									19.5				66		60.7			56.5	

Notes:

SE15
75% P1 R1A4
50/100% P1 ctrl
ctrl
PacBlue CD14/Zombie NIR
30-40% P1/20 vesicular

Thaw cells, DNase, count.
Collect, count, aliquot cells 3.0E+6 Cells R10 / 5ml polystyrene tube
Bring volume up to 1 ml R10, add 2 ul PI-ACTH and CD107a
Cap and incubate at 37°C for 6 hours

Wash with 2 ml PBS, spin down 1300rpm 6min
800 ul of Live/Dead mix (1:2500) @RT for 15min
Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 6min
Add Host stain mix, incubate @37C for 30 min
Wash 2 ml 5% PBS-FBS 1400 rpm, 6 min

Add TetraScan, incubate @RT for 10 min
Add ColdStain mix, incubate @4C for 30min
Add 300-500 ul 1x RBC lysis for 3 minutes
Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

300 ul BD FACS-Prem, incubate @4C for 20min
(vortex every 10 minutes)

First PermWash: 1 ml PermWash 1500 rpm 6 min
Second Perm Wash: 1 ml PermWash 1500 rpm 6 min

Add Intracellular Stain, incubate @ RT for 40min
First PermWash: 2 ml PermWash 1500 rpm 6 min

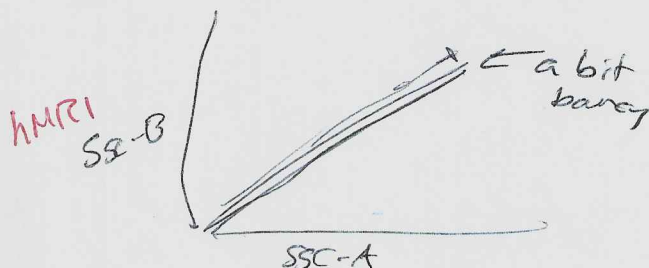
Resuspend in 70 ul 0.4% FACS-FBS
Cap tubes, wrap rack in foil, store at 4°C

2% bdm AF488
12% AF488 CD3

No CD27 in this experiment
clone of BUV80 clone (DX12)

10:50 pm Samples 1st Perm Wash
22:59 pm 2nd Perm Wash
55.5/1 11:10 pm Intracellular @

11:50 pm



Done @ 11:59 pm

Strong TNEa IFNg signal ✓

Avoca PDI Pe-Vo170

4710000 and a bit on activated cells

~~Avoca~~ attempted to on AF647 (Var 7.2 - mislabeled NR1)

CD3 AF647 → debris/dye fields

Small bounce on ~~Zombie~~ CD107a

Set's done @ 7:48ish (6:18 pm)



~ Done 8:52 ish

PMA clearly works

2/1/2023

[illegible]

The issue w/ aggregate tals bright but don't have same AEs.

Zombie ≠ dead cells

TMA & NYCC blooded T cells

Chol vs Fat (gluc in HUS)

Acquisition 6:18 PM

running ~ 600 events/sec Medium ~ 50 p.l? (40 added)

PMT unstained Cold 50 p.l ~ 800 events

CD62L ~ 500 events

1600 ~ 5000 returned

	<u>Udr</u>		CD3	
ND006 Atlantic	46	51.8	22	9
	48	52.2	22	10
ND4062 Atlantic	13%	32% 12%	Viability	13 13
	32%	37%		18 9

No difference in FBS ✓

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