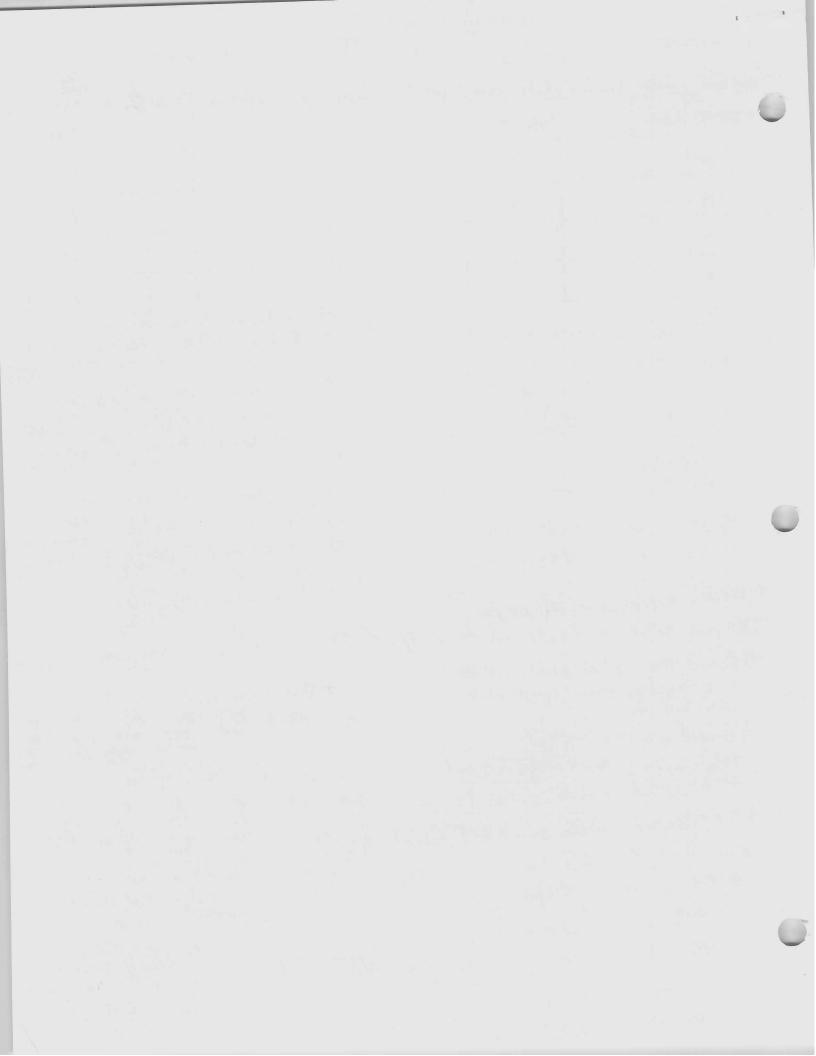
November 22ndh, 2022 Don't Roget to blocks

Monocytes #7

-	Specimen	Status	Location	Conc	Date		Volume	T	1			2 F + 5	
	NDOCE"	Adult		(5EL	11-14-12	Tusks	voiume	Ly	Ly+Mon	Total	3 E+6	2E+6	. 3
				026	() ((-12							316/1	
	(2)											389/1	
	(3)											3641	
	(41											425F1	
	(5)												
	() 25						C) uns	tained for	- S	No. 5	448+1	
	6 ml 23	FBS-	-PBS (+ E07	Staned for samples adlbone (BV711 FoERIA + BV510 CD64									
	2ml +	+ Pac	Blue s	DEC 6+ RD	13)								
							~	The state of the s	ellbone +	_		0.020	
	1	200 pl E	DIA /16	5	7	21	0	y Bac	ilbane +	PeDaze	t Peral	र्देश्य + हैं। 2012 ≥	80 80
		10 + 40 FB 40	inls PBS	\		7 9 30 7.1 3.0							50
	9			.4		3.0	Ø.	Bac	abone +	P= De.	226 L	CD153	
			tmb Zo				Q)	Bar	Whone +	Pelle 2	3	AR-Fire	750
			Smls R							e	03	604	
	3:47 pm	MD:	stown ->	4:02	2 pm		Cip Cip	Boe	albone .	- Spar	(PLe		
	2. 21 ba	Se's	@ 4° (i	~ (0	80) -	4:27 pm	10	Bac	abone -	. 40	3		
	* Forgo!	the	3/1 of b	boll.	will ac	ld				A CC	003	Grz=/180	(
		ners st	- 10 min	period	uhle		7	- Vead	controls at a				
	remedie	2 64:	19 pa - The	:340/					st coces c	0123 6 209955	78-E-	FERT O	
	letra	Mxc5 (@ 4:27	-75	:07pm	$n\int$	~~	\$< co.	ntols (7x.	3)=[2.	750	FERCH @ BUYZI FEE BUT	111
HS	ad Scist T	yse cel	(30/3 ()	4:29 Pr	1->4	32/94	(D)x2	()	0	(D)3	Ø	0	
	Sc's + F.	15 Celled	Morogles	Spira	ed @ [H: 36 pm }	Ser (e Davelo	603 Butu	Sport Sport	APC TIC	CO4 APEFIC 750	
		2, C 9	or mono	eytes	Sa./ -	· -	101	tracci	np Bends	~1,	drop		
			obluce 2	· Ilpm				all	gof 1.24	121	ab tes	velum	
	5 . 48	! C man	ayksat	C 5.2	7 Am,	\$in@5:	31	\ -	30 West 2	5 min (9 4°C	<u> </u>	
				- Made	c1 (0) =	Spines: 5.54 pm	£6:24	pon		400-60	09 3-	FC 5 mn 4% PEA PE	
	K.A.	558 -	= 63	Pen	Top In	Trated the	ie Scls		3.	eadsc	7:00	4% PFA PE	3
	100	Dr 4°C	Spin @6	:34 pm		Dure @	6:48	pm		Page CE CE CE C	7:36	70	



Part														
Redetitype ctriname Ruorochrome Ruoroc		62.7		53.6				Jie	erre ai aw volume/samp	100				
	425	50	425	50					otto draw volume (Dina	8 !!!	D CONTROLS	NSTAINE	nd U
	116.4	13.7	39.1	4.6					Antibody Total Brilliant Stain					
	4.25	0.5	4.25	0.5					True Stain FcX		5			
Per Proposition Proposit			8.5	1.0			(1112)	2000						
Property	#VAL	<1.2>					(CTILL)	CD38	APC/Fire 810		Cells	li.	R8	20
Particular Par	NY N				<u.></u.>			CD123/CD3/CD4	APC/Fire 750		Cells	100	R7	25
Part			8.5	T.0	Agran		(202)	L/D	Zombie NIR		Cell	112500	R6	19
Pubme Pub	8.5	1.0					(5(2)	CD40	APC-R700		Cells		74	5
tere (u)) Referritype ctri name Fluorochrome Marier Clone Validatif Aboder Janna 200 cRP (RPT) 43.2 Accord 20min 40.2 20min 40.2 5.3 40.2 40.2 40.2 5.3 40.2 40.2 5.3 40.2 5.3 40.2 5.3 40.2 5.3 40.2 5.3 40.2 5.3 40.2 5.3 40.2 5.3 40.2 5.3 40.2 40.2 5.3 40.2							(GHI/61)	CD163	Alexa Fluor 647		OH.	E	2 2	1 5
Part (u) Ref crit type ctri name Fluorochrome Marker Clone Vallott Mayor Ma	2.5	0.3			-†- -+-		(1000)		APE		(A)		곱	
The control of the per control			******				(REASOE)	HLA-DR	PE-Vio770		Cells	1135	813	6
Part Cont Part Part Cont Vial Cont			######################################					CD123	PerCP-Cy5.5		200	1 6	DTO BTO	1 2
rer (ul) Ref ctrl type ctrl name Fluorochrome Marker Cone Vial Lot # 30c/RR1 for 30c/RR1	#VAL	<0.6>	7 75	Э			(IT2.2)	CD86	PE-Cy5		- G	1.65	210	3/
rer (ul) Ref crit hype crit name Fluorochrome Marker Clone Vial Lot # 300/Rm 300/Rm 6.7 2 BUV395 CD18 (6.7) Ual Lot # 300/Rm 300/Rm 6.7 5 AF AF AF CD2 CD3 C	.u.	0.4			- - - +		1 1 1 1 1	CD3	PE-Dazzle594		0		8 8	i b
err (ul) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # Blocker Joyco 300min 3	#VAI	<1.2>			-}		(55/1)	CD88	PΕ		(A)	138	B4	14
er (u) Refct/thype ctrl name Fluorochrome Marker Clone Vial lot # Blocker augment 30C (RT) for 30min 65.2 40C for 30min 2 1.33 6.33 CR2 (E132.1D9) Usl lot # 30min 5.1 40C for 30min 6 4.45 AF AF AF (E132.1D9) Usl lot # 30min 5.1 40C for 30min 9 BUV496 AF AF (E132.1D9) Usl lot # 40 0.6 5.1 40 1 BUV561 CX3CR1 (2A9-1) Usl lot # 40 5.2 0.6 6 BUV805 CD14 (M5E2) Usl lot # 4.2 1.2 1 BUV805 CD14 (M5E2) Usl lot # 4.12 1.0 1 BV430 CD64 W5E2) Usl lot # 4.12 4.12 1 BV450 CD64 (B-14) Usl lot # 4.12 4.12 1 BV750 CD64 (ω.	0.4			-			CD3?	Spark blue 550		38		B3	22
er (u)) Refctritype ctriname Fluorochrome Marker Clone Vial Lot # Shocker 30c/RT) for 30min 6.5 4 cfcr 30min 2 3.2 4.5 6.13 (6.7) 4 oc fcr 30min 5.1 4 oc fcr 30min 7 4 4.5 4.1 4.5 4.5 4.1 4.5 4.1 4.1 4.1 4.1 4.1 4.1 4.1 4.1 4.1 4.1 4.1 <td>10</td> <td>1.2</td> <td></td> <td></td> <td>+-</td> <td></td> <td></td> <td>CD11h</td> <td>FITC</td> <td></td> <td>Cells</td> <td>1.28</td> <td>82</td> <td>1</td>	10	1.2			+-			CD11h	FITC		Cells	1.28	82	1
er (u) Ref ctrl type Ctrl name Fluorochrome Marker Clone Vial Lot # 30C (RT) for 30min 6.2 2 1.2 1.2 1.0 1.0 1.0 30min 30min 40.5 40.5 40.5 40.5 40.5 40.5 40.5 5.1 40.5 5.1 40.5 40.5 5.1 40.5 40.5 5.1 40.5 40.5 5.1 40.5 40.5 5.1 40.5					- -			CD141	BV785		Cells		1	1
Cu	10	1.2							BV750		Cells	13		
Cu	00	1.0			- - - -		(0-140)	FcFR1a	BV711		Cells	1.25		11
Cul) Ref ctrl type Ctrl name Fluorochrome Marker Clone Vial Lot # 30/Ckr 570 C(RT) for 6.5	00	1.0			-		(S.LHI.)	CD11c	BV650		2/190	(A)		10
Cul) Ref ctrl type Ctrl name Fluorochrome Marker Clone Vial Lot # 300 cker 370 c(RT) for 6.5 BuV395 CD18 (6.7) Vial Lot # 30/rxn 30min 30min 40 cfor 30min BuV496 Af	12	1.5			- -		(500)	CDEG	BV605		Cells	1.25	V10	9
Cul) Ref ctrl type Ctrl name Fluorochrome Marker Clone Vial Lot # 30/Ckm 570 (RT) for 6.5 BuV395 CD18 (6.7) Vial Lot # 30/min 30min 40Cfor 30min BuV496 Af	10	1.2			<u> </u>		(300)	CD16	BV570		Cells	1.25	V8	00
Column C					+-			CD64	BV510		Cells	pos to	٧7	7
Column Fluorochrome Marker Clone Vial Lot # Side Si	10	1.2			1			20000	BV480		Cells	1.25	V5	T
Ref ctrl type ctrl name	#VA	<1.0>		<1.2>	-			CD66h	Pacific Blue					6
(u)) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # 370C (RT) for 37	8	1.0			<u> </u>		(M5E2)	CD33/CD90	BV421				V1	21
(u) Refctritype ctri name Fluorochrome Marker Clone Vial Lot # 370C(RT) for 8.7 40C for 30min BuV395 CD18 (6.7)	1	1.2						CDIA	BUV805		Celts	1.25	UV16	5
(u) Refctri type ctri name Fluorochrome Marker Clone Vial Lot # 370C (RT) for 8.7 40C for 30min Buv395 CD18 (6.7) 0.6 5.1 40C for 30min Buv496			8.5	1			(2A9-1	CD18	BUV737				U14	4
(u) Refetritype ctri name Fluorochrome Marker Clone Vial Lot # 3JOC(RT) for 8.7 40C for 30min Buv395 CD18 (6.7) 0.6 5.1 40C for 30min Buv496 (6.7) 0.6 5.1 5.1 5.1 5.1 5.1 5.1 5.1 5.1 5.1 5.1								CVOCVA	BUV661		Cells	126	UV11	w
(u) Refetritype ctri name Fluorochrome Marker Clone Vial Lot # 370C (RT) for 8.70 80V395 CD18 (6.7) 1 30min 40C for 30min 40C for 30min 5.1 80V496 B0V496 CCD2 (1.00 CD2)	(5)	0.6			<u> </u>	99)	(IS132.11	CCVZ	6UV615				UV10	
(u) Ref ctrl type ctrl name Fluorochrome Marker Clone V/al Lot # 3JoC(RT) for 37oC (RT) for 30min 6.7 4oC for 30min AF AF AF 4F 4F <t< td=""><td></td><td></td><td></td><td></td><td><u> </u>_</td><td></td><td></td><td>CCB3</td><td>BUV563</td><td></td><td>0197)</td><td>4-1 1-2 1-27</td><td>1</td><td>2</td></t<>					<u> </u> _			CCB3	BUV563		0197)	4-1 1-2 1-27	1	2
(u) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # 3JoC(RT) for 37oC (RT) for 30min 6.7 4oC for 30min AF AF AF AF 0.5 5.1					<u> </u>			2	BUV496				UV7	Τ
(u) Refetritype etri name Fluorochrome Marker Clone Vial Lot # 3JOC(RT) for 6.5 BUV395 CD18 (CT) Vial Lot # 3UJ/Rxn 30min 40C for 30min			5.1	0.6			(0.7)	VE.	AF				9An	Т
(u) Refetrl type ctrl name Fluorochrome Marker Close Value 370C (RT) for 6.5	iin	4oC for 30m.		30min	_ _		(C.Z.)	CD18	BUV395		Dells	53	1 UV2	T
Single color Unmixing Uph FC OF	~		8.5 5	37oC (RT) fo	blocker			Marker	Fluorochrome	Unmixing ctrl name	1			3#

Simplified Protocol
Aliquot cells 3E+6 cells/tube

November 22nd, 2022 Monocyte SFC panel #6

Wash with 2ml PBS, 1300rpm, 8min
2500x diluted Zombie NIR, 1E+6/1ml + 3ul Fc blocker at RT]
Wash with 2ml 2%FBS-PBS-2mM 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-2mM EDTA

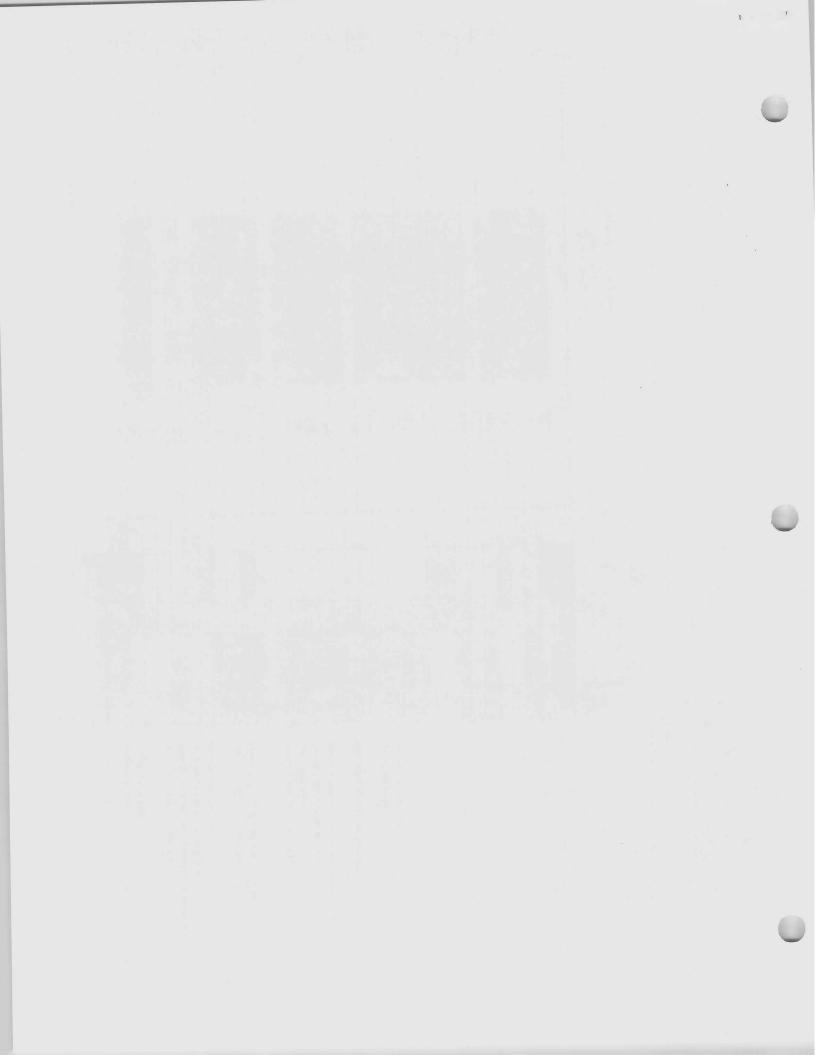
Spin at 1400rpm for 6min

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-2mM EDTA

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



Monocytes SFC Panel

		812		760				697	679	664	613	598	582	542	525	514	508	473	4.58	443	428	388	373	obection
		UV16	UV15	2			UV13	UV12		UV11	UV10		UV9	BVU	04/	;	0		1 / 1	UV4	UV3	UV2	UV1	
		BUV805		DUVISI	BI IV727					BUV661	BUV615		BUV563		DUV496	DI 13 (200	A1-046	1				BUV395		Ş
	1	3		2	1					ವ	<u>3</u>		ವ		[2]							[2]	1	
		CD14		CD19						CX3CR1		í	CCR2		<af></af>		Ą	ļ				CD18		
	0	V16	<15	V14		<u>د</u>	7 7	3	-	V11	<10	§ 8	6	5 6		√5	\$	ప	<u> </u>	ś -	<u> </u>			
			BV785	BV750		04/11	DV744		000	BV650	BV605	0/0/0	BV570	BV510		BV480		PacBlue	I.	-7440	RV421			Violet
		3	3	[2.5]		4	}		[0.0]	3 3	<u>ਤ</u>		[6.1]	2		3		Ξ		3	3		\dagger	
			20144	CD4?		rceR1a	1		CDITC	200	CD#8	CD16	0004					CD66b		CDS!	CPss			
	B14	В13	B12		B11	B10	B9	88	B7	g	3 3	84	В3	B2		B1				1000			\dagger	1
		Pe-VIO//0					PerCP-Cy5.5	PE-Cy5		PE-Dazzie	77	PE	SparkBlue 550	FITC									טומפ	Rijo
		[3]					[2]	[4.5]		[4]		4	3	[1.5]						_			1	
		HLA-DR					CD123	CD86		CD3		CD88	CD3?	CD11b										
100	D X	R7	R6	ā	R 5	R4	R3	R2	R1				25										r	1
0	APC_Fire 810	APC-Fire 750	Zombie NIR		1	APC-R700		AF647	APC														Red	1112212022
3	3	[2]			3	ಪ		[3.5]	[3.5]															
CD36	CD38	CD1237/CD3?	5			CD40		CD163				1												

CD3 CO3

Dazzle Spark Blue/

+ rest pennel

Perco-cys. 5 CO123

BV 750 / ARE-Fred 504 500 COS

11/22/2022

Wednesday 11/23/22 @ 9:37:00: Aurora off (day before thanksgroup), take of contrad? Yz & 1/2 wast/sheather town. Josepha Allen last eser Blu FSC laser has been failing Cash how todo QC on Acronal 2004 lot boods (in the drawer of the type fridge in 7-0274) 300pl Il real like + 1 drop QC bads.

Agains to match MFI % (CV special pedis

16:06 QC on 2004's// Passed V

- Deplicating morocytes taking its time

25,000 Geods

Preth sure c080 + worth on cells

APC-FICTSO looks don? vs other decimels (delfo for CD4, year)

Of the Beads on morocyk schedul

Acquire on low? (didn't run instance)

Sample acquisition @ 11:03 pm 12000 auts/s

Aurora 3 Laser is down Scheduled myself T/R 9:30-12:00 for next 5 weekson 5L

Monogles done @ 12:00pm

Unmixing ~ Nov 23rd, 2022 8 1 attempte COBO BV421 bends \$ CO3 ParPlu CDEES Internal bead controls BU 711 FCERlar & same FSC/SSE worked PERTISS ID123V APC-FICTSO CD123 & CD4&CD3 A tre brightest just bight, or are they junk/doubles (x2 AFI/etc. 15 it poisson noise or something else i what weight the / has? | A Bright or brighted is a lie to CO38* bighter on Lymphocyta (CO40 (swap the negs) Declares my general AF gate impact? CD88 ~ Inled AF production of COSS, HLA-DR, COILC other 2 notable syndres: Leaving as is for now (no tag added); 7 (99.5% your gated events are wall all bused are singlets once gak Wo Tug -> FeeRla blankach (CD80 stan is padled up in Bedls) dorcle 594 16/ CD3 BU421 us PacBlue/CD64

Veg few ossus on Spark Blue 550 PerCP-G5.5 > APT-FIRTSC

ACOH decently behavedthe

e0123

New unmixing AF tags vs Extract + UVG tag

wy or w/o gating brightest vs centered peaks

\$ HLA-DR 0088 FEERS. CDIIC getting band treatment

BUHZI CD80 beads 7
666 beads
FEERIA beads 7
123 Beads
APCFIE750 CD4

CD3 dazzle 123 (35 80BWA)

Ran a sc unstand &? an PacMAP

School my "totration by

Tag in No AF C 0064 for monogle gale
less cors spill I on all gale

fceR1a (beads)

Yestag in Yes AF

= BV421 on? no...

Myeloid Padhop -> no CO3, CO 19, zombie or AF/UVE'S 277, 179 @ 10:31 pm)

700 Eco, oco Trolls
500 200,000 P

