Monocyte SFC panel #2

Specimen	Status	Location	Conc	Data	Tarle	X 7. 1	~	Tu			.3 50	of and
*1	Status	Location		Date OS/12/21	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	2E+6	
NY062×1		_	20EG	08/12/4		2	5.89	7.93		51041	51 pl	RI
NYC6222			20E6	08/12/21		2	6.21	8.32		4834	48+1	17
NY06 223			ZOEF	08/12/21		2	6.83	9.11		4391	44-1	61
NY062 *4			2024	08/12/21		2	7.34	9.76	P 4	40911	HIP	91
7			5.		.a.] [4]		52.5			-1		
Baddhere Chrlsc	0	Total buddben O		-t -t	im boudibon +CD19 -CD20 FEERIN + CD5 - CD141		+00	RIa O	+ CD1 4 + CD1		100 stm/ 10000 10000 10000 10000 10000	a Ela H
J	ostained cells/f		onshmed 36	2-3 st	- 5(n	TX to	N5	3 hrs?		₩	5 kg/m	1
52 5	nstainee	d stained	d vell		differential bughtness confrol	ef	100g	21050 #1 p 50 pm th 16 pm sta	ellet ilw s	şβn	4 .	éη
resord 2. lot set e 4	-3mls 1 -3mls 1 -4:57pm -4:58pm	> 5:15p	יין פאר	2%	FBS 2.0% 2.5% 3.3% > 5% 10	ita/	\$ Ab ₩ Y ×.1 ₩ 44	13. 2 ml (15+1)	4 pm C 2: Reag Reag	13 pm nb prep 3:17 cc 3:25pm	Celb sone	Jam
totses	pn @ 5.74 p	05 pm/	zen S	an 1: 6mn x. 12.0	5 800 000 ml	12 pl 5	-m1 4,	7 121 28	l	To spin	24:05p	21

21 hotrooph

E-34 hot out.

Se Coclect The 36 = 39/40

Het AFAed @ 5:36 pm

Cold Spin@5:42.

Telem adding test thes FOET (best e streeth above report FERTY, CDM)

Streeth adding test these FOET (best e streeth above report)

Streeth adding test these FOET (best e streeth above report)

Cold Ac samples 7.6 5:49 pm (streeth) & He man

Cold Ac samples 7.6 5:49 pm (streeth)

Sob organized @ 5:58 pm

Samples The lyse 6:70 z

To:44 pm Dany moved v findse to emegery

Pomi

1								910	1000					
Market Market Clone Validat Market Clone Validat Market Clone Validat Market M	120	50	/00						ette draw volume/sam	Pinr				
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# Files Color Unmaking Filocochama Marker Clare Validat Marker		0.5	7	0.5					True Stain FcX					
File Single color Lumaling Luncatione Marker Cone Con			14				(ніт2)	CD38	APC/Fire 810				8	21
# Files (m) Red cettilyse citi anne (b) Red cettilyse (citi anne (b) Red cett			14				(10.1)	CD64	APC/Fire 750		F)	15	R7	20
# Files Single color Ulmining Elevations Ulmining Ulmining Elevations Ulmining U					<1:2500>			L/D	Zombie NIR		hu	12	8	13
# Files Single color Unmaking Harris Unmaking Harris Unmaking Harris Unmaking Harris Unmaking Harris Unmaking Harris Unit Harris Harris			14	1.0			(5C3)	CD40	APC-R700		190		R4	18
Single Color Ulmmining Elucochrome Marker Clone Vial Lot # Bode Sack (RT) for 14 Action 30min Marker Clone Vial Lot # Bode Sack (RT) for 14 Action 30min Marker Clone Vial Lot # Bode Sack (RT) for 15 Action 30min Marker Clone Vial Lot # Bode Sack (RT) for 15 Action 30min Marker Clone Clone Marker Clone C									Spark NIF 685				23	
Place Color Colo		1.0					(GHI/61)	CD163	Alexa Fluor 647	0	Ě	Ei Ei	R2	17
Prince Color Col		0.0							APC		(e)		2	
Filter (u) Ref ctrl hype Ctrl name Ruya95 Cione Vial Lot # 3u//ran 30min 20 40 40 40 40 40 40 40		0.5	į				(REA805)	HLA-DR	PE-Vio770	9	Mex	H	B13	16
Single color Unmixing Hiber Hourschirome Marker Clone Vial tot # BUDSA 300/fm 14 300/fm 40 300/fm 40 300/fm 40 400/fg 40 	-		28	2			(7G3)	CD123	PerCP-Cy5.5	0	3115	14.	B10	15
Filter (u) Ref ctrl Nype Ctrl name Fluor chrome Narker Clone Vial Lot # BUO445 Clone Clone Vial Lot # BUO445 Clone Clone Vial Lot # Accior 30min Clone Clone Vial Lot # Accior 30min Clone	+		`	Ci			(112.2)	2000	68700 better				B9	
Single color Unmixing Huorochrome Marker Clone Vial Lott 300 Hore 14 doctor 30min UV2 BUV3 EBUV35S CD18 (6.7) Usl Lott 300min 400 30min 400 50min 11.2 400 50min 11.2 400 50min 11.2 400 50min 400 50min 11.2 400 50min 400 5		0.0	7	0.5			(IT7 2)	CD86	PE-Cy5		Cells	1.78	B8	14
Single color Unmixing Fluorochrome Marker Clone Vallot# Blocker 300(RR) 100 H 400 cfor 30min 400 cfor 30min <td></td> <td>o n</td> <td>5</td> <td>0.0</td> <td></td> <td></td> <td>(==) =)</td> <td>CD3</td> <td>PE-Dazzle594</td> <td>0</td> <td>0.00</td> <td>ij.</td> <td>B6</td> <td>13</td>		o n	5	0.0			(==) =)	CD3	PE-Dazzle594	0	0.00	ij.	B6	13
Single color Unmixing Huorochrome Marker Cone Vial Lot # LOP HE (2017) (2018) 14 40 Cfor 30min UV2 BUV395 CD18 (6.7) Uislot # 30min 30min 40C for 30min UV10 BUV395 CD19 (6.7) Uislot # 30min 40C for 30min UV10 BUV695 CD19 (6.7) Uislot # 30min 11.2 UV11 BUV691 CX3CR1 (2A9-1) Uislot # 10 40 11.2 UV16 BUV695 CD14 (MSE2) Uislot # 11.2 10 V2 BUV11 FERTA (MSE2) Uislot # 11.2 10 V2 BUV395 CD14 (MSE2) Uislot # 11.2 10 V2 BU496 Pacific Blue CD5 (H1111) Uislot # 1.0 1.0 V3 BV520 CD54 (3G8) (3H11) Uislot # 1.5 21 V10 BV520 BV520 </td <td>-</td> <td></td> <td>8.4</td> <td>0.6</td> <td></td> <td></td> <td>(S5/1)</td> <td>CD88</td> <td>PE</td> <td>6</td> <td>6/4 EH</td> <td>i i</td> <td>84</td> <td>12</td>	-		8.4	0.6			(S5/1)	CD88	PE	6	6/4 EH	i i	84	12
Single color Unmixing Hunching Marker Clone Vial Lot H BUDGER 300 min 14 acc for 30min 11 acc for 30min 12 acc for 30min 12 acc for 30min 12 acc for 30min 12 acc for 30min 14 acc for 30min 12 acc for 30min <t< td=""><td>10</td><td>cio</td><td></td><td></td><td></td><td></td><td></td><td></td><td>Spark blue 550</td><td></td><td>CELL</td><td></td><td>В3</td><td></td></t<>	10	cio							Spark blue 550		CELL		В3	
Single color Unmixing (u)) Fluorachrome crit name Marker Clone Vial Lot HUP FR BUILDIAN 14 44 Acc for 30min 14 UV2 BUV395 CD18 (6.7) US 30min 30min 40C for 30min 40C for 30min 11.2 40C for 30min 11.2 40C for 30min 40C for 30min 11.2 40C for 30min 40C for 30min <td< td=""><td></td><td>0</td><td></td><td></td><td></td><td></td><td></td><td>CD11b</td><td>FITC</td><td></td><td>560</td><td>T_a</td><td>B2</td><td>11</td></td<>		0						CD11b	FITC		560	T _a	B2	11
Single color Unmixing cit/l type cit/l name Fluorochrome Marker Clone Vial Lot # 370C (RT) for 30min 14 UV2 BUV395 CD18 (6.7) 30min 11.2 30min 11.2 20min 20min 20min 20min 20min 20min 20min<	L								STSBINGNIBOLY STSBB	-			81	
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Single color Unmixing (ul) Fluorochrome Marker Clone Vial Lot # Up +FC Sungin 44 UV2 BUV395 CD18 (6.7) Vial Lot # 3u/fron 3u/fron 11.2 UV7 BUV395 CD19 (5.7) US 11.2 UV10 BUV496 CD19 (5.1) US 11.2 UV11 BUV563 CCRZ (5.132.1D9) US 11.2 UV14 BUV563 CCRZ (5.132.1D9) US 11.2 UV16 BUV561 CX3CRI (2A9-1) 1 14 UV19 BUV562 GD14 (M5E2) US 1 14 UV16 BUV865 GD14 (M5E2) US 1 14 V2 BUV510 (dim) CD5 (M1111) US 0 0 0 V3 BV510 (dim) CD56 (5.1H11) US 0 0 0 V10 BV650 CD14 (M127)<			21	1,5			(207.4)	CCRS	BV750		Cells	135	V14	10
Single color Unmixing Fluorochrome Marker Clone Vial Lot # 300/Km 300/Km 300/Km 14 UV2 BUV395 CD18 (6.7) Will Lot # 300/Km 300/km 11.2 UV7 BUV395 CD19 (6.7) Will Lot # 300/km 11.2 UV10 BUV395 CD19 (8.132.1D9) 0.8 11.2 UV11 BUV395 CSGR1 (2A9-1) 1 1 14 UV14 BUV395 CD14 (M5E2) 1 1 1 UV1 BUV395 CD14 (M5E2) 1 1 1 V2 BUV395 CD14 (M5E2) 1 1 1 V2 BUV395 CD14 (M5E2) 1 1 1 V2 BUV396 CD15 (H111) 1 1 1 V3 BUV396 CD14 (M5E2) 1 0 0 0 V3		T.U	3	'n			(13077)	CDSO	BV711		2610	35.1	V13	9
Single color Unmixing Fluorochrome Marker Clone Vial Lot # Vial Lot # Sumin 14 blocker 370 c(RT) for		1.0					(BLIVE)	CD11s	BV650	6	celia	100	V11	∞
Single color Unmixing (u)) Fluorochrome Marker Clone Vial Lot # 3u/ran 44 UV2 BUV395 CD18 (6.7) 3u/ran 4u 4u </td <td>8</td> <td>2</td> <td></td> <td></td> <td></td> <td></td> <td>(5 1H11)</td> <td>CD56</td> <td>BV605</td> <td>9</td> <td>all the</td> <td>125</td> <td>V10</td> <td>7</td>	8	2					(5 1H11)	CD56	BV605	9	all the	125	V10	7
Single color Unmixing Fluorochrome Marker Clone Vial Lot # 300/km 300/km 14 UV2 BUV395 CD18 (6.7) 4 300/km 11.2 UV7 BUV395 CD19 (6.7) 4 0.8 11.2 UV10 BUV496 CD19 (15132.1D9) 0.8 11.2 UV11 BUV563 CGR2 (15132.1D9) 0.8 11.2 UV14 BUV661 CX3CR1 (2A9-1) 1 1 UV16 BUV865 CD14 (M5E2) 1 1 V2 BUV865 CD14 (M5E2) 1 1 V2 BUV865 CD14 (M5E2) 1 1 V3 BUV865 BV421 FCER1a (5) 0 0 0 V3 BV480 CD14 (H1111) 0 0 0 0 V3 BV480 CD14 (H1121) 0 0 0 <		7.0					(000)		ofluor 610 better				V10	
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Single color Unmixing LUP+FC LUP+FC LUP+FC LUP+FC LUP+FC LUP+FC LUP+FC LUP+FC LUP+FC Marker Clone Vial Lot # 300min 14 UV2 BUV9553 CCD19 (Is132,1D9) O.8 11,2 UV10 BUV561 CX3CR1 (2A9-1) 1 14 UV16 BUV805 CX3CR1 (2A9-1) 1 14 UV16 BUV805 CD14 (MISE2) 1 14 V2 BUV805 CD14 (MISE2) 1 14 V2 Pacific Blue CD5 (HI111) 1 4 Pacific Blue CD5 (HI111) 14	1	MALI	5	0				COZO	BV510 (dim)	9	SEIIs		5	25
Single color Unmixing LUp+FC LUp+FC LUp+FC LUp+FC LUp+FC LUp+FC LUp+FC BUX955 CD18 (6.7) Up (6.7)		0.6					(HI111)	CD11a	BV480			113	5 5	5
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Single color Unmixing Fluorochrome Marker Clone Vial Lot # Blucker 3700 (RT) for 14		1.0					(141202)	FcER1a	BV421	0				23
Single color Unmixing Fluorochrome Marker Clone Vial Lot # Blucker 370C (RT) for 14 Clone Vial Lot # Clone Vial Lot # Clone Vial Lot # Clone Vial Lot # Clone Cl		,					(MSE2)	CD14	BUV805	9	119.	(4-10 (5-10 (5-10)	UV16	4
Single color Unmixing Fluorochrome Marker Clone Vial Lot # Blucker 370C (RT) for 14			14				(1,0,1)	or work	. £52.4119				U14	1
Single color Unmixing Fluorochrome Marker Clone Vial Lot # Blucker 370C (RT) for 14			2	4			(249_1)	CX3CR1	BUV661		SIMO	No.	UV11	ω
Single color Unmixing Fluorochrome Marker Clone Vial Lot # Bluv363 CGR2 Marker Marker Clone Marker Clone Marker Marker Clone Marker Marker Marker Marker Clone Marker Marker Marker Clone Marker Marker Clone Marker Marker Clone Marker Marker Clone Clone Marker Clone Marker Clone			7.11	0.0			(10102.100)	00101	STAVAB				UV10	
Single color Unmixing Filter (u) Refettitype ctrl name Fluorochrome Marker Clone Vial Lot # 3ul/xn 30min Uv2 BUV395 CD18 (6.7) 14 14 14 15 14 15 15 14 15 15			1	000	1		(16132 100)	CCB3	BUV563		Cell	1.0	6AN	2
Single color Unmixing 1.0 + FC 1.4			7.7.7	0.0	-		(011)	CD19	BUV496	9				22
Single color Unmixing Fluorochrome Marker Clone Vial Lot 300 (RT) for 14	1310		11 2	0.8			(67)	CD18	BUV395		Selection	Ī,		_
				37oC (RT) for	blocker	Vial Lot #	Clone	Marker	Fluorochrome	Unmixing ctrl name	1	Single color (ul)		#

500 pt media ~ 2.5 ps PPO ~/5 ps/ one

Simplified Protocol 3EC Cells/the

2500x diluted Zombie NIR, 1E+6/1ml + 3ul Fc blocker at RT for 15min Wash with 1 ml PBS, 1300rpm, 8min

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

Spin at 1300rpm for 8min

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

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

Spin at 1200 pm for 8min

Spin at 1200 pm for 8min

CDIA FUNC BE NOT WELL

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

Teat with 600ul of 1x Lysing solution at RT for 3min

Teat with 600ul of 1x Lysing solution at RT for 3min

Teat with 600ul of 1x Lysing solution at RT for 3min

Teat with 600ul of 1x Lysing solution at RT for 3min

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

Teat with 600ul of 1x Lysing solution at RT for 3min

Teat with 600ul of 1x Lysing solution at RT for 3min

Teat with 600ul of 1x Lysing solution at RT for 3min

Teat with 600ul of 1x Lysing solution at RT for 3min

Teat with 600ul of 1x Lysing solution at RT for 3min

Teat with 600ul of 1x Lysing solution at RT for 3min

Teat with 600ul of 1x Lysing solution at RT for 3min Treat with 600ul of 1x Lysing solution at RT for 3min ?

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

Resuspend in 0.4%PFA-PBS Spin at 1300rpm for 8min

SQ not quite

(1) final 0.1ug/ml for 24hr Lipopolysaccharides from Escherichia coli 0111:B4 LPS stock 5mg/ml, 10ul; take one aliquot out and add 490ul R10 to make 100ug/ml working solution Wash with 2mM EDTA

InvivoGen tirl-3pelps

Notes: TrueStain FcX (Biolegend 422301)

	L								613												Spectrum	4
	UV16 BUV805			UV14 BUV737		UV13	UV12	UV11 BUV661	UV10 BUV615		UV9 BUV563	UV8	10	UV7 BU V496	UV6	UV5	UV4	UV3	UV2 BUV395	UV1	VU	Monocytes SFC Panel
	[1] CD14			[3]				[3] CX3CR1			[3] CCR2			(Z) CD19		3			[2] CD18			Panel
0 22 A	V16	V15 BV785		V14 BV750	\(\frac{1}{2}\)	V12 BV711		V11 BV650			1	V7 BV510	S	V5 BV480		V3 PacBlue	V2	V1 BV421			Violet	
More cyte Lympiecytes a Lympiecytes a	П	[3] CD141 E		[2.5] CCR5		CDon		[3.5] CD11c			CD16	Tusi CD20		[3] CD11a		[1] CD5		[4] FcER1a				٧
intense	B14	B13 • Pe-Vio770	B12		B10	B9 • PerCP-Cy5.5			B6 PE-Dazzle			B2 4 4 FIIC	-	B1					•		Blue	n rat God for a
extractional extraction	>	[3] HLA-DR			The same	[2] CD123			[4] CD3	1		[1.5] CD11b					3	*				DC modery
Co		7	R6 Zombie NIR	3	APC-R700		R2 AF647	R1 APC													Red	9/26/2022
		[2] CD64			[3] CD40		[3.5] CD163	[3.5]														8

0.72

Aurora-UV early AM edition: butten ran on @ 5:12 AM Shifted time 6:30-9:30 Am, mill adjutaccordingly.

Template set up done @ 5:40 AM, Start@ 5:45 Am Scis - 30,000, median event rote ~ 700 ~ 1 minuted (ymphogks) remember monocytes today lare, (smalar)

PE C088 in Pole's solve to decorpling? Thest vin export se as fes and unmix in Extra Tools.

Samples start @ 6:30 am 1 Smin each

Maying short-tim gains paying long team consequences Hovid Huascines

CO19, CO3, CO56 unmikedur hym AF, everything else monogk.

ItLA-DR Pe-Viozzo almost off scales

CDIA AF signal ~ longliked Boell on Buveas

BV570 CD16 brightest might be an Wils ~ monocytes ~ 1051 (Few 16+ monocytes in this donor)

BUTIL CD80 #@ 3 hrs stim posture leading edge negative + great control

CCRS driblets 1051 exagerated

HLA-DR high expression (10 ~ 10 ~

18100

COSO Minimal 11

FEERTA maggiral most cos 104 edge ~ ccrs spoth? N CO 141 2 0086 ACD123

64/46

A Monogle us DC/PDC express the GH/HO expression axis A May need to draw new AF gate Erb \$ swap out colo AF for cos Did 16 get added? 18 darar some?

Not c03/2:

CDB + CDII c collb

corz collb

CDEH CD38

CKSCRI CONS

CDEY 0038

COIY . collb

0084

64 38

Dila . Cale

collb

64

CO16. CAZER1

·Colle

CON