9:10 AM PPD@ 11:26 am # 20 Ec N406 2 08 12 21 PMA @ 11-36 an MON DONS. HalgBlack 1.48 pm 10:08 stan for count (CUD4282, NYU60, NYU6222) got dropped, san .. may undercant? CUD 300 C/Pl 20pl 5E6 ~ 10E6 Skephoal ARC. 4.03EH 4.53EH4 N4060 380 C/Pl 10.8 pl Z 6.80 E4 8.85 E+4 18.42pl 2 4.49E4 6.05 E+4 13.16pl ABS Z 6.19E4 8.32 E+4 2N 201 ZOL ZOL ZOL WOSt C unst unst 8.06 -7 00 00 0 0000 13.60 00 00 [5M] 00 00 8.98] - 21.36 007 on stards 0-0. _ 007 2 H CD1072 O.Vazz O THUS O , 372 £5 4/1.424M/W 2220 1.5/5.34 = .280 unstored, ~ 130pl 130 yl 280 yl

3,1 +95 182 800 V

01/03/2022 Colline Mor and test AB/Mon CUD4782 5/ O 10/12/20 2 N4060 06/03/21 000 At temporaris NYOGZ than for telegimen test \$ 12 well instead of 6 Polystyrene plates for today.... 20:39 por stain Zoun Canle 8:50pm = CND4282 490e/pl 1.5/ml 19ml 11.43 (1.2 mls) ~ 8.7 M = 8.45 Eq 11.3 EG 2 wells (tight fit) 1130 NYCGO SQUENT 8.65 E6 = 7.03 E 6 [2ml] 13.5 17.3 KZ Ince 9:11 pm 3 wells (tight F.H)

Tetramer Staining Order

January 4th, 2023

		And UNSTA			27	26	25	24	23	138	22	21	2 2	3 5	5 5	10	17	16	15	14	28	13	12		11	10		9	00	7	6	•	. .	4	w r	2	1	H #
		And UNSTAINED CONTROLS !!!			R8	R7	R6	R4	R2	RI	813	810	*	0	1	0 0	20.0	87	VIS	V14	V13	V11	V10		V7	VS	V3	V3	VI	UV16	U14	UVII	OATO	LW10	LIV9	IIV7		Filter Single color (ul) Ref ctrl
					•					4							718000	-	•		• .	1	ø.		•	•	•	•	•	•								Unmixing ctrl
				APC/Fire 810	APC/FIRE /50	Combie NIR	APC-R/00	ABC BZOO	AlexaFluor647	APC	PE-vio770	PerCP-Cy5.5	PE-Cy5	PE-CF594	PE	Spark blue 550	AlexaFluor 488	BV786	BV750	TT/A9	Dione	BVGEO	BV605		BV510	BV480	Pacific Blue	Pacific Blue	BV421	BUV805	BUV737	BUV661	BUV615	BUV563	BUV496	AF	865AN9	Fluorochrome
				CD38	CD27	L/D	CD107a	COLUMN TO A STATE OF THE PARTY	hMB1	CD16	PDI	TNFa	CD25	CD26	NKG2D	CD3	hCDId	CCR6	IFNY	CD7	CCK/	Cons	CDSS	40.000	CDASEA	CD161	CD19	CD14	CD127	CDA	CXCR3	V82	CCR4	CD69	CD8	AF-UV6	CD62L	Marker
/sample	Pippette draw volume	R10 Media	Antibody Total	(HIT2)	(0323)		(H4A3)				No.	(MAB11)	(M-A251)	(M-A261)		(SK7)		(11A9)	(B27)					(minor)	TECHNI	(CEACAR)	(DESID)	(MSE2)	(A019DS)	CXCR3)	(106/	(86)	(161)	(FN50)	(RPA-T8)		(DREG-56)	Clone
100		1	2				N					1																										Vial Lot#
19.5		-	2.0				2.0			-	-	-	-	-												1												During stim!!!
Pipp	777	3	24			<1:	24			+	1	1	1	1	-	-							-	-			-	-	+		-	1						12
Pippette draw volume /sample	brilliant Stain	Brillians	Antibody Total			<1:2500>																																L/D 15 min (RT)
ıme /sample	tain		Total					<: >()									<: >()																					Tetramer 40 min @ RT
67	50		19.5	16	2							1.2	7.2	1.5	1			15		1	1.5	1.0			2			1.5	1.5			2.0						HotStain 30min @37C
	600		234	19.2	24							14.4	14.4	18	3			18	;	12	18	12			24			18	18			24						n 12
60.7	50.0	1	13.7						0.7	1.5					1.2									0.7		2.0	2.0	・しつらん		2.5	0./	1	0.5	0.1	2	1.2	10	ColdStain 30min @4C
	600	1	164.4					13	8.4	18.0					14.4									8.4		24.0	24.0			30.0	8.4		6.0	8.4	2	14.4	1	12
																											•	2										RBC Lyse, then Fix/Perm
54.5	50	13	7 5							0.5		0.5		0.5				1.5		0.0	On								0.5				1					Intracellular Stain 40 min @RT
	600	2	3							6.0	30.0	6.0		6.0				18.0		0.0	0								6.0				12.0					12

Simplified Protocol

Thaw cells, DNAse, count.
Collect, count, allquot cells 30E+6 Cells R10 / 5ml polystyrene tube
Bring volume tubor in R10, add 2 ut PNAVCtrl and DuringStim antibody
Cap and incubate at 37°C for 6 hours

Wash with 2 ml PBS, spin down 1300rpm 8min 800 ul of LiveDead mix (1:2500) @RT for 15min Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 8min

Wash with 2 ml PBS, spin down 1300rpm 8min Add tetramers for 40 minutes at RT

Add HotStain mix, incubate @37C for 30 min Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

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 FixPerm, incubate @ 4C for 20min (vortex every 10 minutes)

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

Add Intracellular Stain, incubate @ RT for 40min First PermWash: 1 ml Per

1 ml PermWash 1500 rpm 6 min 1 ml PermWash 1500 rpm 6 min

Second Perm Wash:

Resuspend in 100 ut 0.4% PFA-PBS Cap tubes, wrap rack in foil, store at 4°C

NXT 25.26

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