	Specimen	Status	T	T =				1:	100			
	Specimen	Status	Location	Conc	Date	Tasks	Volume	Lv	Ly+Mon	Tr. ()		
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4:38 everything pre-pred 2.0 Fl 4:39 cells out of incubator 5:01 ccar7 in for stain -> 5:31 Telsamers @ 5:29 >6:10 > wash cet @ 6:14

cel 7 5:43 in for Frilliam > done 6:27 ILTTE framer tables into 870 @ 6:25

Cold stan @ 7:11
RR yse @ 7:45pm -> work@ 7:49 Fixfern 7:58

End@ 20:47 pm/

Innate-like T cell SFC panel

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		56.25			7			pie	lime /		William Street							
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Filtry Single-code/(a) Red cut Duminic C			-			3.2						(НІТ2)	CD38	APC/Fire 810				
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Filter Single color Unmining cirt Unmini				2.0			1 2			1		(M-A261)	CD26	PE-CF594				
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Filter Single color (u) Ref ctrl name Fluorochrome Marker Clone UV2 15 min (RT) for 40 min 370C (RT) for 2 2 Surface staining 2 CytoFiy/ lhtra mixRT for 40 min 1.2 2.4	0.50		4000	2.0	1.0							(161)	CCR4	BUV615			OTO	
Filter Single color (u) Ref ctrl name Fluorochrome Marker Clone Vial Lot # Stim!!! UV2 BUV395 CD82 (RPA-T8) UVA During 2 15 min Tetramer 370C (RT) for 2 Stim!!! Tetramer 370C (RT) for 2 Surface staining 2 CVOEIN/ 40C for 30min 40C	0.50	0.25		1.4	0.7							(FN50)	CD69	BUV563			100	
Filter Single color (u) Ref ctrl name Fluorochrome Marker Clone Vial Lot # Stim!!! 2 Js.min Tetramer 37.0C (RT) for 2 Surface staining 2 CryoFixy Intra mix 10.00 CryoFixy				2.4	1.2					-		(RPA-T8)	CD8	BUV496			1/0	
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	2	Intra mixRT for	then CytoFix/	2	Surface staining	2		Tetramer	1	/		Clone	Marker	Fluorochrome	Unmixing ctrl name		1	ì

Simplified Protocol

Add 500 ul of PMA-ionomycin/Ctrl Aliquot cells 2.0E+6 Cells/tube Raise R10 + cell volume to 500 ul Incubate 6 hours @37C

Wash with 2 ml PBS, 1400rpm, 6min

Wash 2 ml 5% PBS-FBS 1400 rpm, 6min Wash 2 ml 5% PBS-FBS 1400 rpm, 6min Add Tetramers, incubate @RT for 40min 800 ul Live/Dead @RT for 15min

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

Add cold Surface mix, incubate @ 4C for 30min Add 300-500ul 1x RBC Lysis for 3 minutes Wash 2 ml 5% PBS-FBS 1500 rpm, 6min

300 ul Cytofix/perm, incubate @ 4C for 20min
Wash twice 1 ml PermWash 1500rpm, 6min

Wash once w/ 2ml Perm Wash Resuspend 100 ul 0.4% PFA-PBS, store 4C Add Intra Staining, incubate @ RT for 40min

