B14 for PF- F. re 810

## JMC AIM Panel - NYOG2 - There SEB for 6 hrs -12/02/22

During   1   U/0.15 min RT   370c for   6   Suring of Continuin   12   12   12   12   12   12   13   14   14   14   14   14   14   14					Autofluorescence
During   1   L/D 15 min RT   390°C for   20   20   20   20   20   20   20   2		volume			· Unstained Controls: 1 for Unstimulated single colors; 1 for Stimulated single colors,
During   1   L/D 15 min RT   370 Cf or   30 min   30 mi	<b>300</b> 50		1	for Sample	
During   1   L/D 15 min RT   370 Cfor   20   20   20   20   20   20   20   2	07		ntibody Total	AFC/IIIE 010	R8
During stmill         1         V/D 15 min RT         370C for stmill         Surface AoC for John in Summary         48°C for Acc Luner intra MRST for Grading         Foot33 min of Intra MRST for Grading         Foot33 min of Intra MRST for Grading         Foot33 min of Intra MRST for Grading         5 min of Grading         48°C for Acc Luner intra MRST for Grading         6 min of Grading         48°C for Acc Luner intra MRST for Grading         6 min of Grading         48°C for Acc Luner intra MRST for Grading         6 min of Grading         48°C for Acc Luner intra MRST for Grading         6 min of Grading         48°C for Acc Luner intra MRST for Grading         6 min of Grading         48°C for Acc Luner intra MRST for Grading         6 min of Grading         48°C for Acc Luner intra MRST for Grading         6 min of Grading         48°C for Acc Luner intra MRST for Grading         6 min of Grading         48°C for Acc Luner intra MRST for Grading         6 min of Grading         48°C for Grading <td><b>87</b> 13.7</td> <td></td> <td>(HIT2)</td> <td></td> <td>R7</td>	<b>87</b> 13.7		(HIT2)		R7
During   1   L/D15min RT   370Cfor   6   Staning   1   S	96	1	(eBio64DEC17)	の日本	R6
During   1   L/D 15 min RT   370C for   6   24°C for   30min   1   15 min   1   1   15 min   1		×1.23002			R4
During stimil!         1         U/D 15 min RT         376 for 30min         6         3 stimil (a and bottom) and bottom (a and bottom) and bottom) and bottom (a and bottom) and bottom (a and bottom) and bottom) and bottom (a and bottom) and bottom (a and bottom) and bottom) and bottom) and bottom (a and bottom) and bottom) and bottom (a and bottom) an			4-	APC-R700 CD107a	R2
During stim!!!   1   L/D 15 min RT   370c for   6   15 min   12   12   12   13   14   14   14   14   14   14   14				AF647	
During stimil!   1   L/D 15 min RT   370°C for stimil!   Average AoC   Coto Sixy   Average Coto Sixy   A			(A1)	APC CD39	P1
During stimil!   1		1.3	(84)	PE-W0770 778 810 0X40	B13
During stim!!!         1         I/D 15 min RY         370C for stim!!         6         Surface 4oC for 30min         4°C for About the for the print of the prin			(BENESE)		B10
During stim!!!!         1         It/D 15 min RT         370c for stim!!!         6         Surface 4oC for 30min         4°C for Adomin         RECURIENT Problem         Intra MINRT for Chologisty         6         For 30min stim           1         1.2         7.2         4.2         0.5         3         1.5         1.5         3         1.5         3         1.5         3         1.5         3         1.5         3         1.5         3         1.5         9         0.25         1.5         3         1.5         1.5         1.5         9         0.25         1.5         1.5         1.5         1.5         1.5         9         0.25         1.5 <td></td> <td></td> <td>(24-31)</td> <td>2000</td> <td>B8</td>			(24-31)	2000	B8
During stimil!   1   L/D 15 min RT   370Cfor   6   11 min   11 m			(M-A251)		B6
During stim!!!   1   L/D 15 min RT   370Cfor   6   11 min   6   12 min   6   15 min   6 min   15 min   6   15 min   15 m	1.2		(M-A261)	PF-CF594 CD26	84
During stimil!   1   L/D 15 min RT   370Cfor   6   15 min   1   12   12   12   13   14   14   14   14   14   14   14		1.2	(EH12.2H7)	PD-1	83
During stimil!   1   L/D 15 min RT   370Cfor   6   Surface 40C   6   15 min   Crefn   P   40 min   Crefn   P   4			(587)	Spark blue 550 CD3	
Duffing stimil!   1   L/D 15 min RT   370C for somin   6			(5/3)		B7 Delta
During stim!!!   1   L/D 15 min RT   370Cfor   6   Surface 40C   6   15 min   Cerim   Cerim			(236A/E7)		V15
During stim!!!   1   L/D 15 min RT   370c for stim!!!   1   L/D 15 min RT   370c for stim!!!   2   2   2   2   2   2   2   2   2		Cort	(11A9)	BV786 CCR6	V14
During stimi!!   1   L/D 15 min RT   370c for somin   6   1.2   7.2		4	(B27)		V13
During stim!!!   1   L/D 15 min RT   370c for solution   30min   6   1.2   7.2			(DX29)	BV711 ICOS	V11
During stim!!!   1   L/D 15 min RT   370c for solution   330n in   6	0		(G043H7)	CCR7 \	VIO
During stim!!!   1   L/D 15 min RT   370c for stim!!!   270c for somin   6   12   7.2		1.5	(125204)	CXCR5	
During stimi!   1   L/D 15 min RT   370c for stimi!   1   L/D 15 min RT   370c for stimi!   1   L/D 15 min RT   370c for somin   6   Staining   15 min Core min RT   40 min   6   Staining   15 min Core min RT   40 min   6   Staining   12   12   13   15   15   15   15   15   15   15	8	2.5			V /
During stim!!!   1   L/D 15 min RT   370c for somin   6			(illian)	CD45KA	V7 CH
During stim!!!   1   L/D 15 min RT   370c for solution			(H:100)		VS
During stimi!   1   L/D 15 min RT   370c for   6   fox 23   15 min   6   15 min   6 min   6   15 min   6 min   6   15 mi				Pacific Blue	V3 Cells
During stimi!!   1   L/D 15 min RT   370c for stimi!!   1   L/D 15 min RT   370c for stimi!!   1   L/D 15 min RT   370c for somin   6   Staining stimi!!   1   L/D 15 min RT   370c for 30min   6   Staining stimi!!   1   L/D 15 min RT   40min   6   Staining stimi!!   1   L/D 15 min RT   40min   6   Staining stimi!!   1   L/D 15 min RT   40min   6   Staining stimi!!   1   L/D 15 min RT   40min   6   Staining stimi!!   1   L/D 15 min RT   40min   6   Staining stimi!!   1   L/D 15 min RT   40min   6   Staining stimi!!   1   L/D 15 min RT   40min   6   Staining stimi!   1   L/D 15 min RT   40min   6   Staining stimi!   1   L/D 15 min RT   40min   6   Staining stimi!   1   L/D 15 min RT   40min   6   Staining stimi!   1   L/D 15 min RT   40min   6   Staining stimi!   1   L/D 15 min RT   40min   6   Staining stimi!   1   L/D 15 min RT   40min   6   Staining stimi!   1   L/D 15 min RT   40min   6   Staining stimi!   1   L/D 15 min RT   40min   6   Staining stimi!   1   L/D 15 min RT   40min RT			(HIB19)		V3
During stim!!!   1   L/D 15 min RT   370c for somin   6   Surface 40C   6   4*C for somin   6   Staining   6			(M5E2)	CD14	V1
During stim!!!   1   L/D 15 min RT   370c for   6   Surface 40C   6   4*C for   NEC UTEN   Intra MixRT for   6   Staining			(A019D5)	CD127	UV16
During stimi!!   1   L/D 15 min RT   370c for   6   for 30min   6   for 30min   6   15 min   6 min   6   15			(SK3)		U14
During stimi!!   1   L/D 15 min RT   370c for   6   Surface 40C   6   4*C for Cycle   New Unit   Intra MixRT for   6   Staining   15 min   Cycle   40min   6   Staining   4*C for Cycle   4*	1.5		(1C6/CXCR3)	CXCR3	UV11
During stim!!   1   L/D 15 min RT   370c for somin   6   Surface 40C   6   4*C for somin   1.2   7.2	18	3.0			OTAN
During   1   L/D 15 min RT   37oC for   5   5   15 min   6   6   6   15 min   6 min   6   15 m			(161)	BUV615 CCR4	089
During stim!!!   1   L/D 15 min RT   37oC for   6   fox 23   15 min   6   15 min   6 min   6   15 min   6 min   6   15 min   6 min   6   15 min	12	2.0	(FN50)	BUV563 CD69	UV)
During stimi!!   1   L/D 15 min RT   370c for   6   Surface 40C   6   4*C for Cycle   New Union   Intra MixRT for   6   Staining   15 min   Cerm   40min   6   Staining   12   7.2   15   15   15   15   15   15   15   1			(RPA-10)	BUV496 CD8	280
Stimili 1 L/D 15 min RT 370c for stimili 1 L/D 15 min RT 30min 6 Surface 40C 6 4*C for cycle intra MixRT for 6 Staining erm / 40min 6 Staining			100	6	240
During 1 L/D 15 min RT 370c for 6 Surface 4oC 6 4*C for CytoFix/P 40min 6 Staining stim!!! 1 1.2 7.2			(KEG 50)	CDBZL	INCO STREET STREET
During 1 L/D 15 min RT 37oC for 6 Surface 4oC 6 4*C for KNEUTRI Admin 6 Staining stim!!! 1 1 L/D 15 min RT 30min 6 for 30min 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1			8	e Walker	Filter Single color (ul) Ref ctrl type name
1 L/D 15 min RT 37oC for 6 Surface 4oC 6 4*C for RE-CURRY Intra MixRT for 6 Fox83 30min 6 for 30min 6 15 min cerm 40min 6 Staining				Marker	Unmixing ctrl
RBC Inen	6 Surface 4oC for 30min				er 23rd 2022 WAULT cell of c parior mine

ICOS, CD40L, OX40, 4-1BB to be added 1.2 mcl/sample at 4C

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Simplified Protocol

Rest cells overnight in 6 \ Collect, count, aliquot cel Add antigens, stim antibo Cap and store incubator a Add golgi block, and incul

Wash with 1-2 ml PBS, 1: 800 ul Live/Dead 1:2500 Wash 2 ml 5% PBS-FBS 1

Add hot Surface mix, incu Wash 2 ml 5% PBS-FBS 1

Add cold Surface mix, inc Add 300-500ul 1x RBC L Wash 2 ml 5% PBS-FBS 1

300 ul Cytofix/perm, incu

First PermWash: Second Perm Wash:

Add Intracellular Stain, in Wash once w/ 2ml Perm

[Fix for FoxP3, Stain for F

Resuspend in 100 ul 0.4%

Cap tubes, wrap rack in fo