

December 20th, 2022

SFC Panels - Cord Blood Test

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	1.0E+6
Inf 692-7 60μl	HU $\frac{1}{2}$ TNG $\frac{1}{2}$ e-1	Box1A1	(a)		bloody	2	3.84	4.98	7.68 (9.96)	2111	
	HU $\frac{1}{2}$ TNG $\frac{1}{2}$ e-2	Box1A1	(a)		bloody	2	4.07	5.41	8.14 (10.92)		
rept	NDO06	Adult	ISEG	11-14-22		2	4.84	6.17	9.68		
no pl		Adult	ISEG	11-14-22		2	5.16	6.60	10.32		

Pulling specimens @ 10:40 am

Thaw cord @ 10:50
11:10 DNase

11:41 am counting...

conc running 1579 c/p/1
18 μl ~ 1000 events

Not adding DNase
to Adult = mistake

Seeds [cord] 3.95 → 759 μl • 2531
adult [] 5.05 → 594 μl • 198 μl

Extras
PM

cord	○	○ ○	○	○
Adult	○	○ ○	○	○ ○

10 μl ~ 1500 → 30x1
X63
1.9 ml

12:51 pm in incubator w/ protein transport inhibitor
- w/ 5μg/ml of Tuberulin PPD
- ILT/AB samples ≈ 3 μl CD107A

4:19 pm → SC tubes labelled (dear mother d. gal)
Ab prep start @ 4:44 pm

hotpacs @ 5:23 prep

6:11 Tcell panel's nearing completion

6:21 pm Tcell panel ab mixes done

< Pausing making monocyte cold @ 6:48 pm >

W/D @ 7:19 pm (filling cold room started keeping)
→ 734

SPM @ 7:38 pm

Vortexed & stored @ 7:50 pm

1/4 Tetrances @ 8:16 pm → 8:56 pm //

Hot 37°C @ 8:22 pm → 8:52 pm

1/4 SC's @ 8:41 → 56 pm

SC cells spin @ 8:48 pm //

-4 extra AF channels ~ cord specific
-4 extra AFG47/CD3 direct for comparison

→ Tets 2 lots wasted @ 9:02
Spin @ 9:08 pm

Cell SC's aliquoted DBVi cd
& placed in bridge
@ 9:12 pm

Hot ILTs/cold Mon & ABs

→ 9:24 pm → 9:54 pm

10:02 SC's into 48 Car
~ 30 min

10:32 →
(not bothering stamp
tonight)

→ Spin @ 22:10

Strept @ 10:25 → 10:40 pm

ILT's cold @ 22:30 → 23:00 pm

Spin streps & SC's (no RBC here)

11/12 SC's for hc Old/hMRE1 in 15 min stain

Cold ILTs spinning

Monocytes & SC's done ✓

AB's + SC's waiting ILTs before FixPerm

FixPerm @ 11:23 pm → 33 → 43 (starting to get ready to prep beads,,)

SC's 486/447 one step behind

*< No Sc streptavidin for BV480 CD61 preFixPerm > *

First PermWash @ 11:53pm

will add @ 40min?

2nd permwash @ 00:11pm

no idea what effect will be.

Intra @ 00:43 am → 1:23 am

absorb → 51 am → 1:31 am

SC's beads @ 1:17 am → 1:47 am

SC Cells + samples done @ 1:46 am

63
× 2
126

Beads + cells fixed @ 2:13 am → 26 → 36 am

Beads Done @ 2:52 am

December 20th, 2022

SFC Panels - Cord Blood Test

	Thaw	6 hrs
Mon	cord Adult O O	O O
ILTs	O O O - O → Tet Controls	O O
AB's	O O	O O

10 million 10 million
↓ 14 million ↓ 14 million

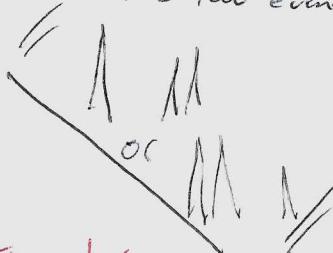
23 Sc's 23 Sc's
40 Sc's 40 Sc's
63 Sc's 63 Sc's
x.2 x.2
12.6 Sc's 12.6 Sc's

Beads
6:50 start //

(fixed beads showing a triplet pattern) ~23,000 cells
((a little over a minute for 10,000 events))
(1:30)

< the rest on the waste is aerosolizing! > [Upps!] @ 12:14 pm vs Cord ILT in unstained slot]

Something happened w/ the fixing
↑ 20,000 events //



? Did I vortex them?

and by 1d2 it was normal.... wtf! (missing has stopped)

Monocyte acquisition sc's @ 60pl
Samples @ 90pl ~ 10:30?

adult monocyte unstained 2400 events ~ 28.62 pL
at MFI ~ 10⁴

* Doubts ~ Indicative of disease
or cell fragility?

Definitely need CD66b for cord,
maybe something else ...

* when the pump goes it glitches *

Cord samples ~ 13000 events / 28 pL
Sample 3:15 sec

Sc's moment of truth events ~ 900
[max 1/3 Lymph & all gates]

150 events/sec (not enough monocytes)
[Lost most CD18n] up to 190 events/sec

Monocytes (cells) done @ 12:10 pm

Sc by last ~ 424 events (55 pL)

CD62L → 170 event/sec

(Ab's lack of vision)

* Apc affects ← * (2511 cells/sec)

(Odd outburst by the flow user in the sorting room)

→ minding my own business idea

Bead acquisition continued on...

Q: Was it the separate batches? strong possibility... and its back... recommended

- Fixing? - overstaining? - batch?

Is it additive on top of AF or dropped/accounted on/off

20:20 Microplate I

Something seriously wrong w/ occurr...

2 Delta bead lots have ΔAF?

Vortex well, beads don't disupt as easily (noticing vs event rate)

Mind last Pre CD16

pretty uneven staining towards the end here

→ may not be conclusive "beads =/= well"

5.3 GB's of Data

84 98 22

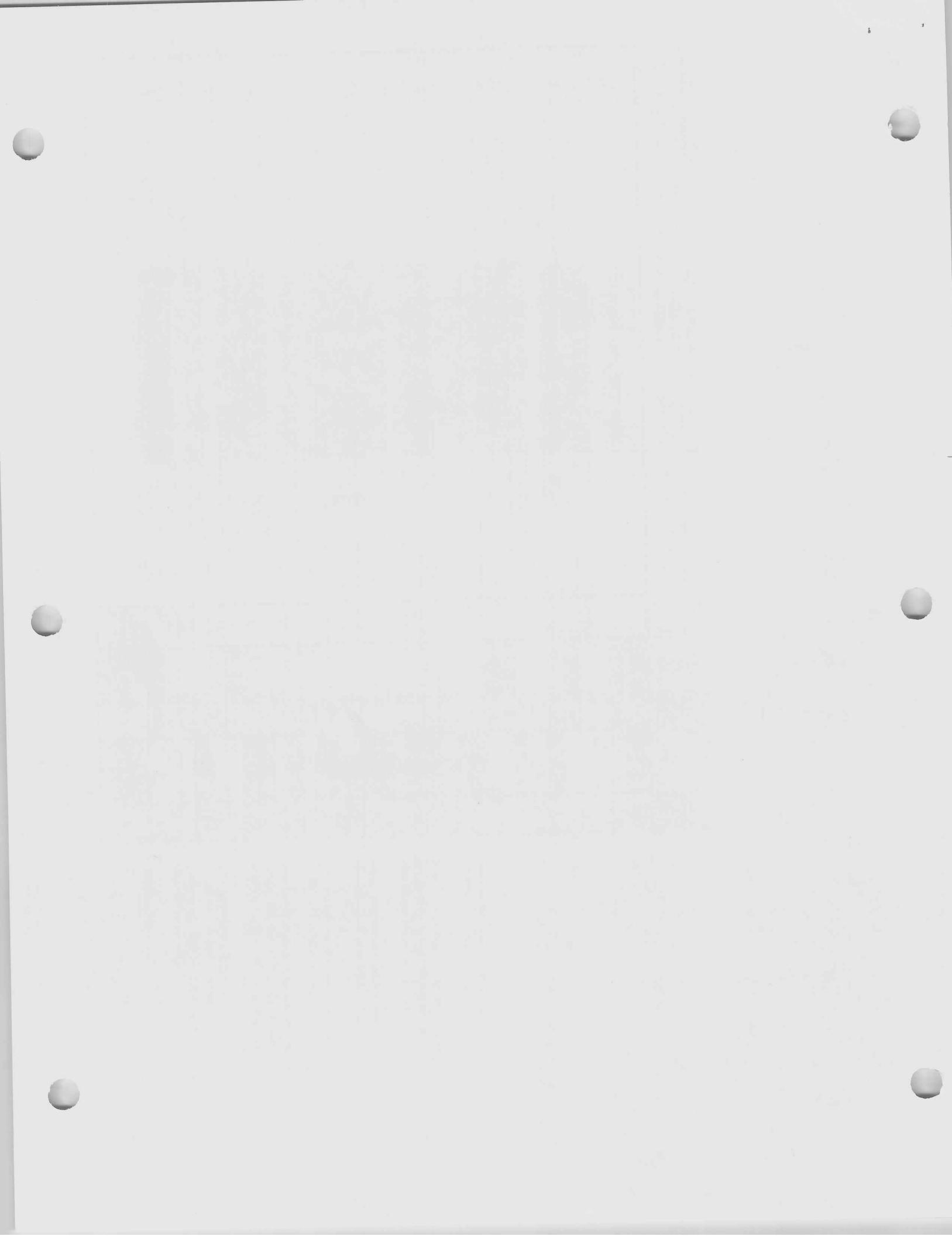
2 3 3

92

116

5

143



#	Filter	Single color (ul)	Ref ctrl	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	During summl	4	L/D	Tetramer:40 min @ RT	4	ColoStain 30min @4°C	4	RBC Lys, then FixPerm	Intracellular Stain 40 min @RT	4
1	UV2				BUV395		CD62L	(DR065-59)							1.2	4.8		
2	UV7				AF		AF-U6											
3	UV9				BUV496		CD8	(BPA5-8)							0.7	2.8		
4	UV10				BUV623		CD99	(BPA5-9)							0.5	2.0	1	4.00
5	UV11				BUV625		CCR4	(1G1)							2.0	8	0.7	2.8
6	UV14				BUV661		V62	(B6)							1.6/			
7	UV15				BUV737		OKR3	(CKR3)							2.5	10.0		
8	V1				BUV835		CD4	(S3A1)							1.5	6	0.5	2.00
9	V3				BUV421		CD427	(AD1975)							1.5	6		
10	V5				Pacific Blue		CD14	(MSE2)							2.0	8.0		
11	V7				BV450		CD161	(HE651)							2.0	8.0		
12	V10				BV510		CD4RA	(H1L00)							0.7	2.8		
13	V11				BV605		CD56								1.0	4		
14	V14				BV650		CD87								1.5	6	0.5	2.00
15	V15				BV750		IFNγ	(B22)							1	4		
16	B2				BV786		CCR6	(1149)									1.5	6.00
17	B3				AlexaFluor 488		hCD14								1.5	6		
18	B4				Sham blue 550		CD3	(SK7)							<1: > ()			
19	B5				PE		NKG2D								1.2	4.8		
20	B8				PE-Cy5.54		CD26	(M-A261)							1.5	6	0.5	2.00
21	B10				PE-Cy5.5		CD25	(M-A251)							1.2	4.8		
22	B13				PerCP-Cy5.5		TNFa	(MA511)							1.2	4.8	0.5	2.00
23	R1				PE-viv770		PD1								1.5	6.0	0.5	2.00
24	R2				APC		CD6								0.7	2.8		
25	R6				AlphaFluor647		hMIR1								<1: > ()			
26	R7				APC-C700		CD107a	(H443)		2.0	8	<1: > ()						
27	R8				APC/Fire 810		CD27	(O33-3)							2	8		
					CD28	(H112)									1.5	6.4		
Anti UNSTAINED CONTROLS II									Antibody Total	2.0	8	Antibody Total	17.5	70	15.7	62.8	7.5	30
Anti Media									R10 Media	18.5	74	Brilliant Stain	50	200	50.0	200	50	200
Pipette draw volume /sample									Pipette draw volume /sample	19.5		Pipette draw volume /sample	65		62.7		54.5	

Simplified Protocol

Tissue cells, DNase, count.

Collect, count, aliquot cells 3.0E6 Cells R10/5ml polystyrene tube. Bring volume up to 1 ml R10, add 2 ul PHA/Ch and DuringSift antibody. Cap and incubate at 37°C for 6 hours

Wash with 2 ml PBS, spin down 1300 rpm 8 min

80 uL of LiveDead mix (1:250) @ RT for 15 min

Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 8 min

Add tetramers for 40 minutes at RT

Wash with 2 ml PBS, spin down 1300 rpm 8 min

Add Hoechst mix, incubate @37°C for 30 min

Wash 2 ml 5% PBS-FBS 1400 rpm, 8 min

Add ColoStain mix, incubate @ 4°C for 30 min

Add 300-500 uL IX RBC-Lys for 3 minutes

Wash 2 ml 5% PBS-FBS 1400 rpm, 8 min

300 uL BD FixPerm, incubate @ 4°C for 20 min

(vortex every 10 minutes)

First Perm/Wash:

Second Perm Wash:

1 ml PermWash 1500 rpm 6 min

Add Intracellular Stain, incubate @ RT for 40 min

First Perm/Wash:

Second Perm Wash:

1 ml PermWash 1500 rpm 6 min

Resuspend in 100 uL 0.2% PFA-PBS

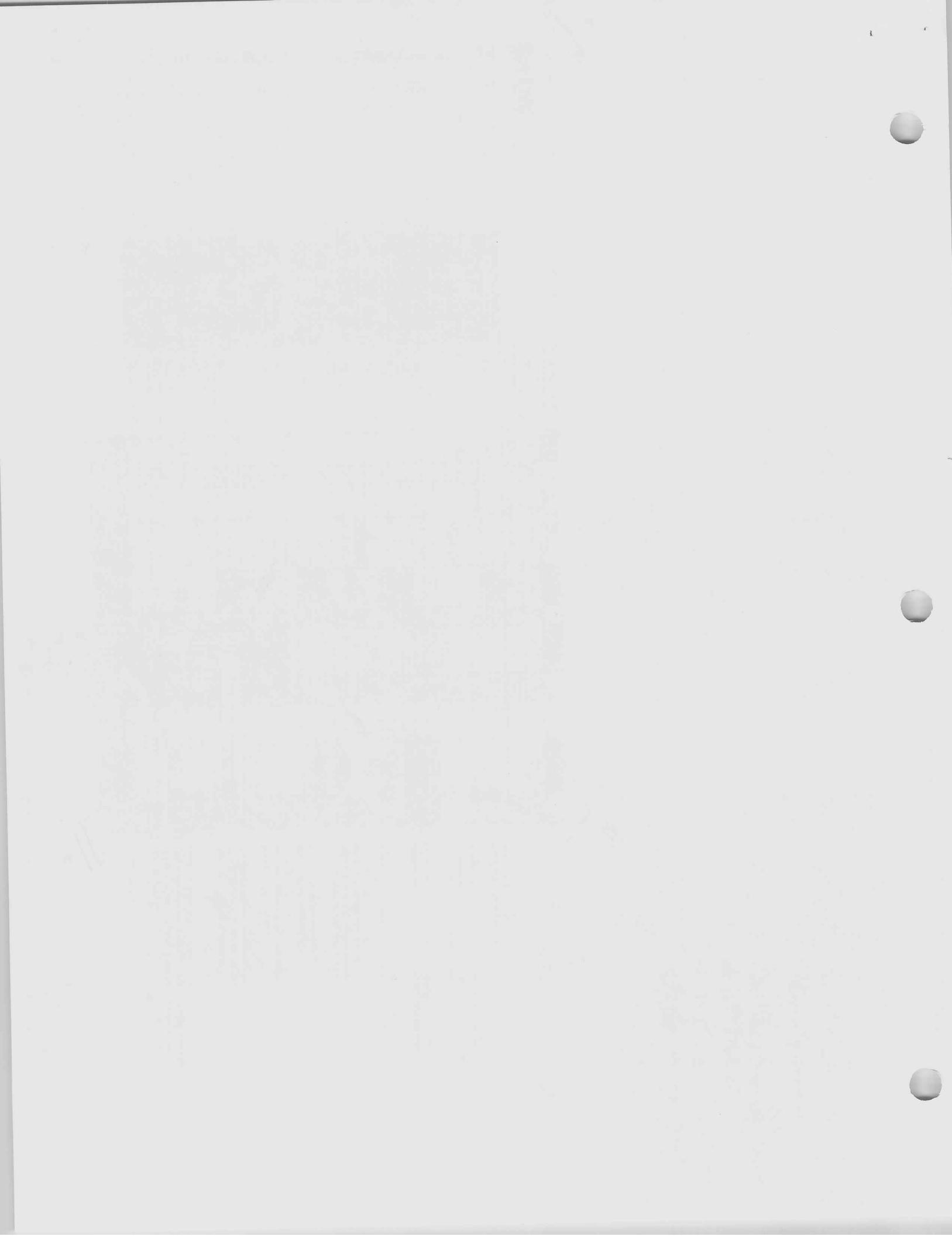
Cap tubes, wrap back in foil, store at 4°C

Notes:

No

CCR23 ~ ch today
~ 12/22/22
161 scrap56/CD21/NKG6/CD167c
Speed

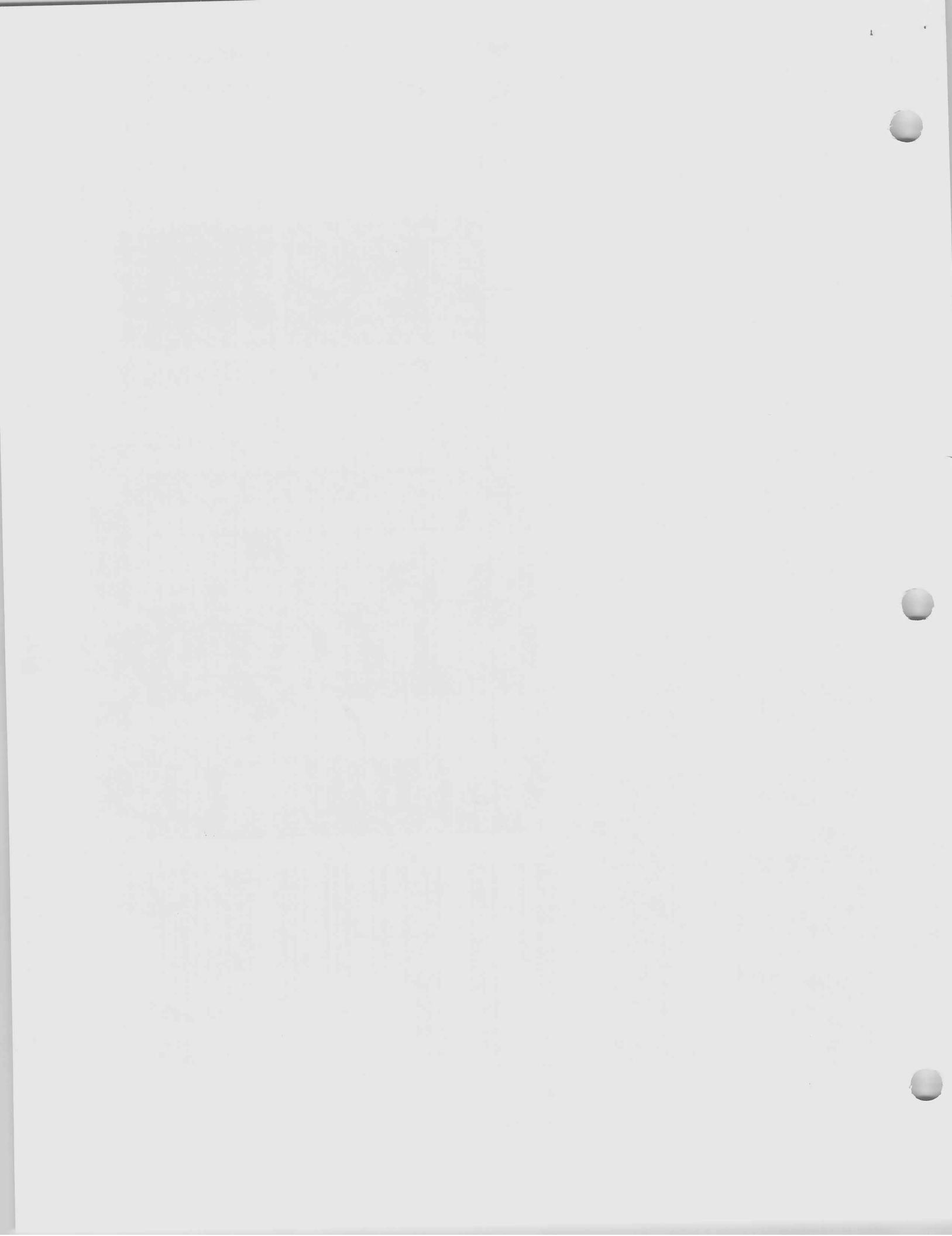
e025 labeled green



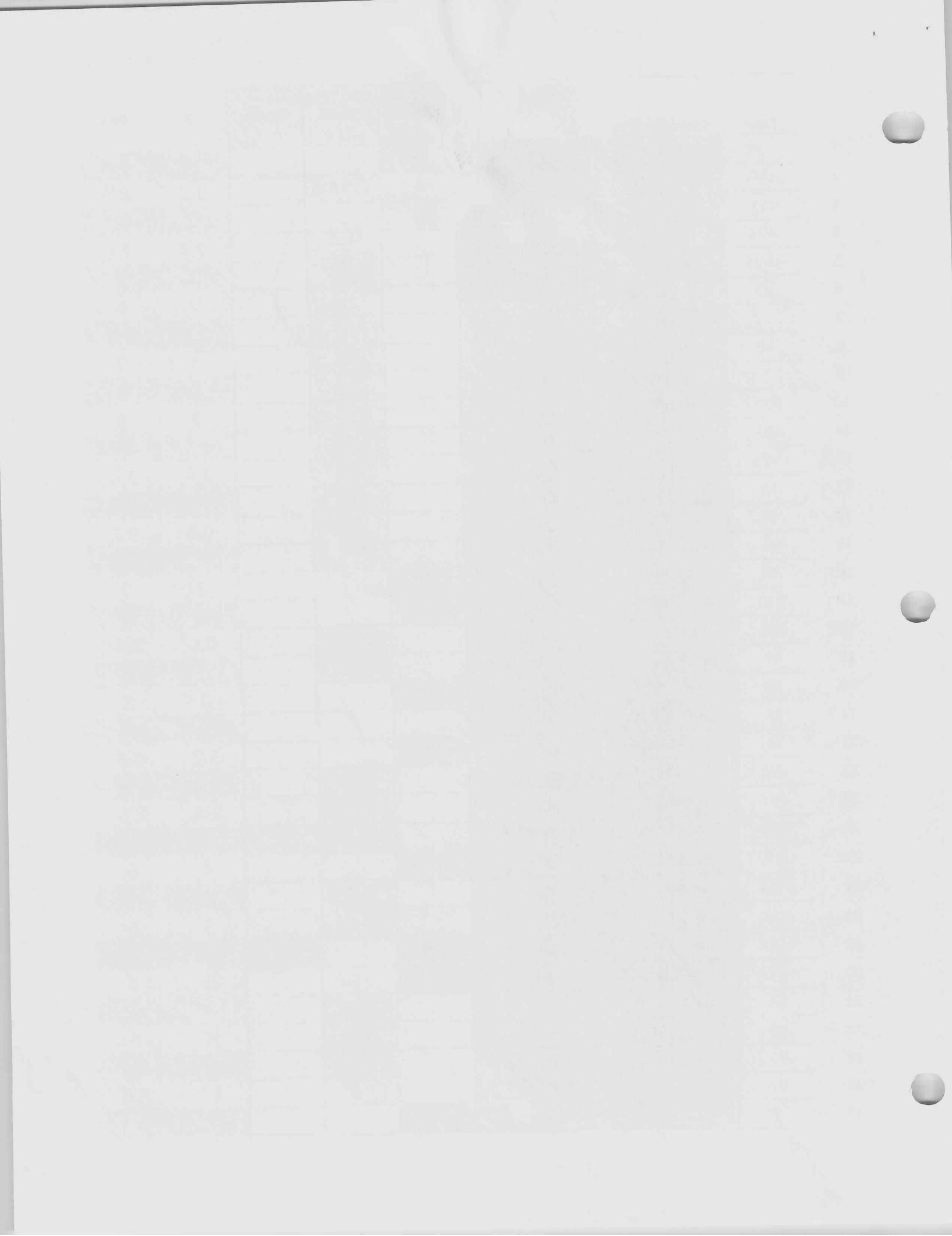
卷之三

Notes:

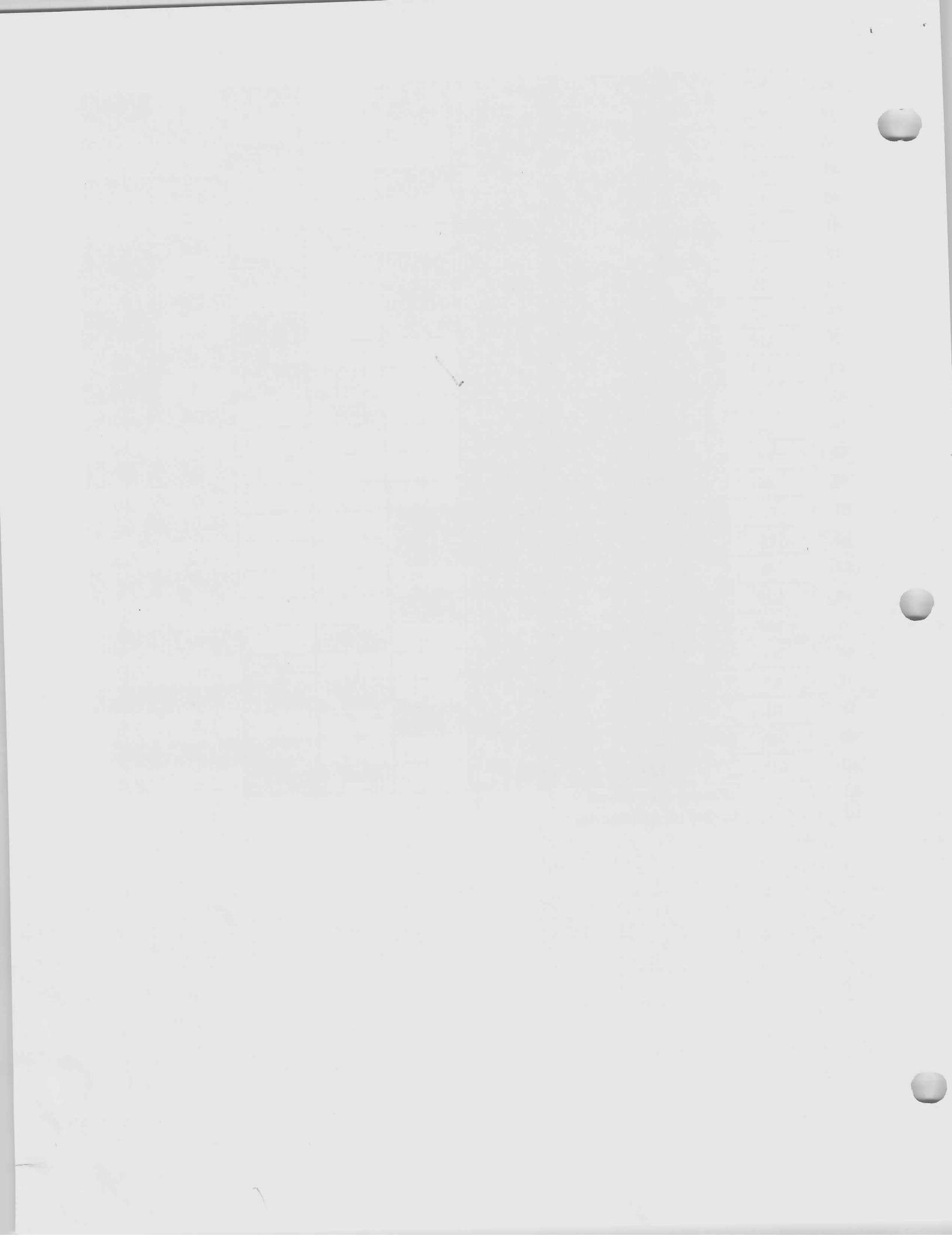
Can tubes wrap rack in foil stores at 41°C



	Filter	Fluorochrome	Marker	HotStain 30min @37C	ColdStai n 30min @4C	Spiked	2	PBS
1	UV2	BUV395	CD18	0.6			1.2	8.8
2	UV9	BUV563	CCR2		0.6		1.2	8.8
3	UV11	BUV661	CX3CR1	1			2	8
4	U14	BUV737	CD19		1.2		2.4	7.6
5	UV16	BUV805	CD14		1.0		2	8
6	V3	Pacific Blue	CD66b		1.0		2	8
7	V7	BV510	CD64		1.2		2	8
8	V8	BV570	CD16		1.5		2.4	7.6
9	V10	BV605	CD56		1.0		3	7
10	V11	BV650	CD11c		1.0		2	8
11	V13	BV711	FcER1a		1.2		2	8
12	V15	BV785	CD141		1.2		2.4	7.6
13	B2	FITC	CD11b		0.4		2.4	7.6
14	B4	PE	CD88		0.4		0.8	9.2
15	B6	PE-Dazzle594	CD3		0.6		0.8	9.2
16	B8	PE-Cy5	CD86	0.5			1.2	8.8
17	B10	PerCP-Cy5.5	CD123	2			1	9
18	B13	PE-Vio770	HLA-DR		0.3		4	6
19	R2	Alexa Fluor 647	CD163		1.0		0.6	9.4
20	R4	APC-R700	CD40	1.0			2	8
21	R6	Zombie NIR	L/D				2	8
22	R8	APC/Fire 810	CD38	1.0			0	10
23	UV2	BUV395	CD62L		1.2		1.2	8.8
24	UV7	BUV496	CD8		0.7		2.4	7.6
25	UV9	BUV563	CD69		0.5	1	1.4	8.6
26	UV10	BUV615	CCR4	2.0			3	27
27	UV11	BUV661	Vδ2		0.7		4	6
28	U14	BUV737	CXCR3		2.5		5	5
29	UV16	BUV805	CD4	1.5		0.5	4	36
30	V1	BV421	CD127	1.5			3	7
31	V3	Pacific Blue	CD14		2.0		4	6
32	V3	Pacific Blue	CD19		2.0		4	6
33	V5	BV480	CD161		2.0		4	6
34	V7	BV510	CD45RA		0.7		1.4	8.6
35	V10	BV605	CD56	1.0			2	8



36	V11	BV650	CCR7	1.5		0.5	4	36
37	V13	BV711	CD7	1			2	8
38	V14	BV750	IFN γ			1.5	3	7
39	V15	BV786	CCR6	1.5			3	7
40	B2	AlexaFluor 488	hCD1d				0	10
41	B3	Spark blue 550	CD3		1.2		2.4	7.6
42	B4	PE	NKG2D	1.5		0.5	4	36
43	B6	PE-CF594	CD26	1.2			2.4	7.6
44	B8	PE-Cy5	CD25	1.2		0.5	3.4	30.6
45	B10	PerCP-Cy5.5	TNF α			2.5	5	5
46	B13	PE-vio770	PD1		1.5	0.5	4	36
47	R1	APC	CD16		0.7		1.4	8.6
48	R2	AlexaFluor647	hMR1				0	10
49	R4	APC-R700	CD107a				0	10
50	R6	Zombie NIR	L/D				0	10
51	R7	APC/Fire 750	CD27	2			4	6
52	R8	APC/Fire 810	CD38	1.6			3.2	6.8
53	V5	BV480	CD161 -Biotin				0.3	10.1
54	V10	BV605	CXCR5	2.5			5	5
55	V13	BV711	Va7.2		1.2		2.4	7.6
56	B3	FITC	FOXP3/Vd1			<4>	0	10
57	B4	PE	PD-1		1.2	0.5	3.4	30.6
58	R1	APC	CD39	1.2			2.4	7.6
59	R2	Alexa Fluor 647	IL2			1.2	2.4	7.6
60	B13	PE-vio770	HLA-DR			0.8	1.6	8.4
61	Unstained Monocytes							
62	Unstained Lymphocytes							



Monocyte & DC SFC Panel		12/20/2022			
Spectrum	UV	Violet	Blue	Red	
373	UV1				
388	UV2	BUV395	[2]	CD18	
428	UV3		V1	BV421	
443	UV4		V2	[4]	
458	UV5		PacBlue		
473	UV6	AF-UV6	[1]	CD66b	
508	UV7	BUV496	[2]	<AF>	
514			V5	BV480	[3]
525			V4		
542	UV8		V6		
582	UV9	BUV563	[3]	CCR2	
598			V7	BV510	[1.5]
613	UV10	BUV615	[3]	BV570	CD64
664	UV11	BUV661	[3]	CD16	B3
679	UV12		V9	SparkBlue 550	[1]
697	UV13		V10	PE	[4]
717			V11	CD11c	B4
738			B6	FITC	B2
750	UV14	BUV737	[3]	CD56	B7
760			V12	PE-Dazzle	B7
783	UV15		V13	CD3?	[4]
812	UV16	BUV805	[1]	BV785	
			V14	BV750	[2.5]
			V15		
			V16	CD14	B14
				Pe-Vio770	[3]
				HLA-DR	R8
				Zombie NIR	R6
				APC-Fire 750	R7
				APC-Fire 810	R5
				CD163	R4
				CD40	R3
				APC-R700	R5
				CD647	R1
				AF647	R2
				CD163	[3.5]
				CD40	[3]
				L/D	
				CD38	



100%
Affect tet is 0.95
Strong evidence for my theory
(if my way subtraction is correct)



$$R_1 - R_2 \approx 0$$

Spectrum	UV	Violet	Blue	Red
373	UV1			
388	UV2			
428	UV3	[2] BUV395	[2] CD62L	
443	UV4		BV421	[4] CD127
458	UV5		PacBlue	[1] CD14/19
473	UV6			
508	UV7	[2] BUV496	[2] CD8	V5 BV480 [3] CD161
514	UV8			V6 FITC [1.5] Vd1/FoxP3
525	UV9	[3] BUV563	[1.5] CD45RA	V7 BV510 [1] CD3
542	UV10		V8 BV570	V8 PE [4] PD1
582	UV11	[3] BUV615	V9 CCR4	V9 PE-CF594 [4] CD26
598	UV12	BUV611	V11 BV650	V11 CCR7 [3.5] CD25
613	UV13		V12 BV711	V12 PerCP-Cy5.5 [4.5] TNFa
664	UV14		V13 Va7.2	V13 APC-R700 R4 AF647 [3.5] CD39
679	UV15	[3] BUV737	V14 BV750	V14 APC-Zombie NIR R5 IL-2 [3.5] IL-2
697	UV16		V15 BV786	V15 APC-Fire 810 R6 R7 CD38 [3] CD107a
738		[1] CD4		
750				
760				
783				
812				
			[3] HLA-DR	R8 L/D CD27 CD38



PacMAP AFs started @ 1:50 pm for 2.4 M cells (400K each adult, 800K cord) comparing overall fix/no fix. ~Is the no space between islands result of total cells ~ AF continuum? If I did fewer, what?

// Starting a better folder management, the sheets that make this paper document will be included in the folder of analysis.
Plan eventually include unmixed & raw files into it, zip it and store.

Beads unmixing cells (Monocytes) (Universal bead negative control)

Pe-Ni.0770 → Poly7 for today

CCR2 B: $10^{5.12}$ peak C: _____

CD3 B: $10^{5.12}$ peak C: _____

CD11b: B: $10^{5.23}$ peak 20%
 $10^{4.12}$ peak 80% C: _____

CD11c: B: $10^{5.12}$ peak C: _____

CD14: B: $10^{5.01}$ peak 50%
 $10^{4.14}$ peak 50% C: _____

CD16: B: $10^{5.01}$ peak 20%
 $10^{5.00}$ peak 60%
 $10^{4.12}$ peak 30% C: _____

CD18 B: 10^5 peak 40%
 $10^{4.12}$ peak 60% C: _____

CD19 B: $10^{5.12}$ peak 60%
 $10^{4.14}$ peak 40% C: _____

CD38 B: $10^{5.01}$ peak 66% C: _____
 $10^{4.01}$ peak 33% C: _____

CD40. B: $10^{5.01}$ peak 90% C: _____

CD56. B: $10^{5.12}$ peak C: _____

CD66b B: $10^{5.01}$ peak C: _____

CD86 B: $10^{5.12}$ peak C: _____

CD88 B: $10^{5.23}$ peak
 $10^{5.10-1}$ peak C: _____

CD123 B: $10^{4.14}$ peak
~
 10^4 peak C: _____

CD141 B: $10^{5.11}$ peak C: _____

CD163 B: $10^{5.13}$ peak C: _____

B: 10^5 peak C: _____

(no negative bead peak...) hint of a suppeak

Cx3cr1: B: $10^{5.11}$ peak C:
 $10^{4.04}$ peak

FcER1a B: $10^{5.13}$ peak C:

HLA-DR B: $10^{5.12}$ peak C:
 $10^{4.12}$ peak

CD64 ~ adult being used for cell unmoving beads over cord example.

CD3, CD11b,

Some CD16, some CD18, 40, 5% HLA-DR

Low CD19, Low CD38,

Cells ~ Monocytes ~ peak 50

~ most cell controls didn't show many monocytes... ad hoc?

Will this extra tools approach work?

^{AB}
Initial T cell tracking: Beads, strep BV480 AF extract, w/ cells Zambi. NFR
universed negative beads (and CD27 cells)

L.J.'s point on screenshots your SDS was a good point.

Were monocytes not fixed issue?

CD3: B: $10^{4.12}$ C: ✓

HLA-DR B: $10^{5.11.2}$ C: ✓

IL-2 B: $10^{5.12.3}$ C: ✓

CD62L B: 10^5 60% C: ✓

B: $10^{4.12}$ 40%

CD8: B: $10^{5.11.2}$ C: ✓

CD69: B: 10^6 70% C: ✓

B: $10^{4.12.7}$ 30%

CCR4 B: $10^{5.12}$ 20% C: ✓

B: $10^{4.12.3}$ 80%

Vδ2 B: $10^{5.13.4}$ C: ✓

CXCR3 B: $10^{5.12.8}$ C: ✓

CD4 B: $10^{4.12.8}$ C: ✓

CD127 B: $10^{5.12}$ 50% C: ✓

B: $10^{4.12.2}$ 50%

CD14 B: 10^5 C: ✓

ZD61 (stop) B: $10^{4.14.5}$ C: ✓

CD45RA B: 10^5 C: ✓

CXCR5 B: $10^{5.14.5}$ 20% C: ✓

W_{4.12} 30%

CCR7 B: $10^{5.11.2}$ C: ✓

Vα2 2 B: $10^{5.12}$ C: ✓

IFNG B: $10^{5.12.2}$ C: ✓

CCR6 B: $10^{5.13}$ C: ✓

B: $10^{4.12}$

VD1 B: $10^{5.13}$ C: ✓

PDI B: $10^{5.12}$ C: ✓

CD26 B: $10^{5.11}$ 50% C: ✓

B: $10^{4.12}$ 50%

CD25 B: $10^{5.13}$ 20% C: ✓

B: $10^{4.13}$ 90%

TNFα B: $10^{4.14.5}$ C: ✓

CD39 B: 10^5 C: ✓

CD102A B: $10^{5.11.2}$ 40% C: ✓

W_{4.12} 60%

CD38: B: 10^5 C: ✓

CD27: B: $10^{5.12}$ C: $10^{4.12}$

B: 10^4

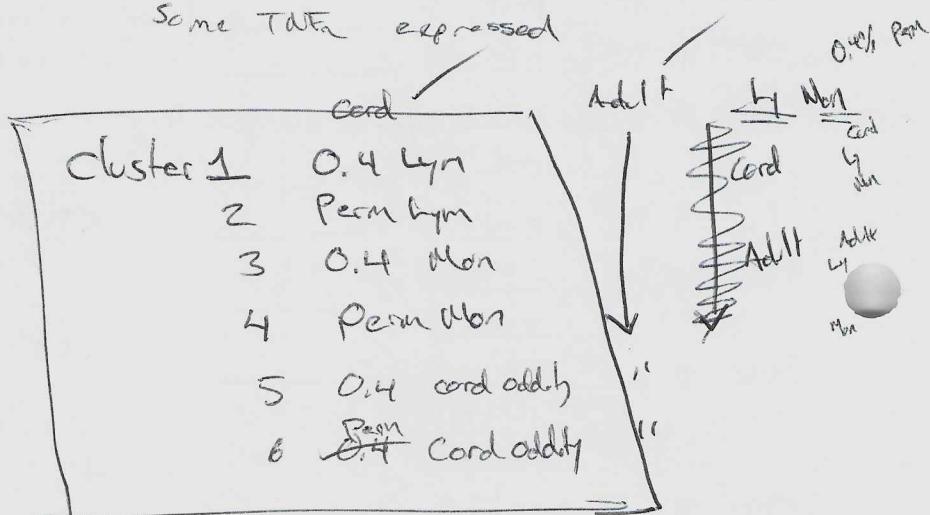
e. K strong suspicion 2 types of beads

HLA-DR mostly fine except for brightest
CD8 suffer

VD2 ≠ not good

CD161 same issues w/ stop

Some TNFa expressed



So... per my markdown

At normalized cosine similarity for Lym⁺

(1 & 3) Fresh Cord & Adult 1.0

(2 & 4) Permed Cord & Adult 1.0

(1 & 2) Fresh Cord vs Fixed Cord 0.95

(3 & 4) Fresh adult vs Fixed adult 0.96

→ same condition
cord & adult have
equivalent AFs

→ Δ condition AFs ≠

At normalized cosine similarity for Mon⁺

(1 & 1) Fresh Cord & Adult 0.99

(2 & 4) Fixed Cord & Adult 1.00

(1 & 2) Fresh cord vs Fixed cord 0.97

(3 & 4) Fresh adult vs Fixed adult 0.99

the above predominantly
holds

At normalized cosine cord

(1 & 3) Fresh Ly⁺ & Fresh Mon⁺ 0.99

(2 & 4) Fixed Ly⁺ & Fixed Mon⁺ 1.00

(1 & 4) Fresh Ly⁺ & Fixed Mon⁺ 0.94

(2 & 3) Fixed Ly⁺ & Fresh Mon⁺ 0.98

At normalized cosine adult

(1 & 3) Freshly & Fresh Mon⁺ 1.0

(2 & 4) Fixed Ly⁺ & Fixed Mon⁺ 0.99

(1 & 4) Freshly & Fixation 0.94

(2 & 3) Fixed Ly⁺ & Fresh Mon⁺ 0.91

December 20th, 2022

SFC Panels - Cord Blood Test

Monocyte subtypes is 0.93-0.96 simbly 0.97-0.98
Checking Monocytes #6... →

Bonus parameter got involved ~ no name into the
Screen. Poors correlation...
in term

Ab cell mix → TNF α , IL-2 & IFNs by bced
Zombie NFR, strip BV480, AF extract CD4 Panel

AB cells ~ comp

006? high flying stingers /CCR4/421

CXCR3

CCR7 is screened up.

At all CD3 level:
PackMAP no AF,
no Dimp
no Zombie

Minimal comp needed for AB panel.

900,000
@ 4:56 pm
12/26/2022

ILT cell unmixing - Need to remove FITC
no need to switch up gates, woohoo!! Computer noticeably slower
now running PackMAP

Ab CD8 Vdkz con 1a1 VDV/plat appendix

CD4's: 582,000

ILT comps:

? BV480 cage coming apart?
CCR7

→ second round of PackMAP

ILT PackMAP @ 5:55pm
for 1.3 Million Cells

Monocytes (dreading this unmix... ??)

38 64 CDR8 X23
Zombie CCR2
HOT CDR4
HCKDR CCR5
CD43 ZB
CD45 FCR4L
CD46 CD4T
CD51 ab

Taking home 7GB
Solder

PackMAP 2 90,000 cells

No CD19, CD66b, CD3, L/T or AF

6:57pm

