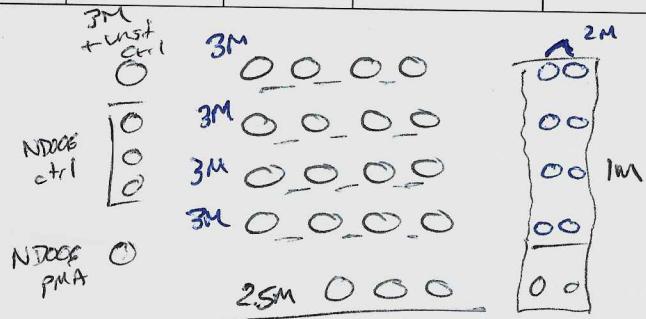


May 12th, 2023

2023_ILT_01

Specimen	Status	Location	Conc	Date	Notes	Volume	Lym	Lym+Mon	Total
2100pm Inf182-1 a-s 595G	HU ♂	Box 6A (4) F9	(5.4)			1.5	9.47	12.9	14.2
1740pm ab 595T			(6) ca. 7			1.5	5.06	7.33	7.59
1130pm Inf281-1 a-s SLMC	HEU-10	Box 8A (3) B3	(12.9)			1	6.78	11.2	11.20
1150pm ab SLM D			(5)			1	6.39	11.0	11.00
1600pm Inf052-7 a-3 RVSC	HEU-hi	Box 3A H.5 (B)	(5.6)			1.5	10.5	16.0	15.75
1640pm ab 594V	HU ♂	Box 6A, C.8 (7)	(7.47)			1.5	10.1	16.9	15.15
ND050	Adult		(1)			1	9.62	13.3	9.62
ND006	Adult		(2)			1	8.24	10.2	8.24

Start 8:26 am
8:43 2nd set
9:03 DNase
9:40
9:42 start for count
Count done @ 10:14 am
11:22 unstained gelized
11:35 PMAs



11:38 am Intracellular "start"
Incubation
→ 5:38 pm

Main antibody mixes & reagents
done @ 5:33 pm

5:44 pm cells spin
5:59 pm samples worked
6:08 pm SC spin
6:26 pm w/ 750µl

6:38 Samples spin

6:44 SC 2 ambic → 7:00 pm

6:57 pm 37°C SC → 22 Spin 7:30

7:02 pm 37°C samples → 33

7:12 pm → 7:42

8:08 pm Tetrabers in Abs prepped @ 7:23

TiKPerm SC @ 8:13 pm → 23 → 33 A (Spin @ 8:40)

8:19 pm ABS in → 8:50 pm

Leftovers for all 4 specimens
(4, 8, 2, 2 EE respectively)
given to Dr. Rothnau

Cold 4°C samples 9:22 → 9:52 pm

9:32 Intra SC → 10:12

9:38 SC stored

RBC lyse @ 9:57

Spin @ 10:04 pm

Samples Fix Pm (10:20 → 30 → 40)

1st spin @ 10:41 pm

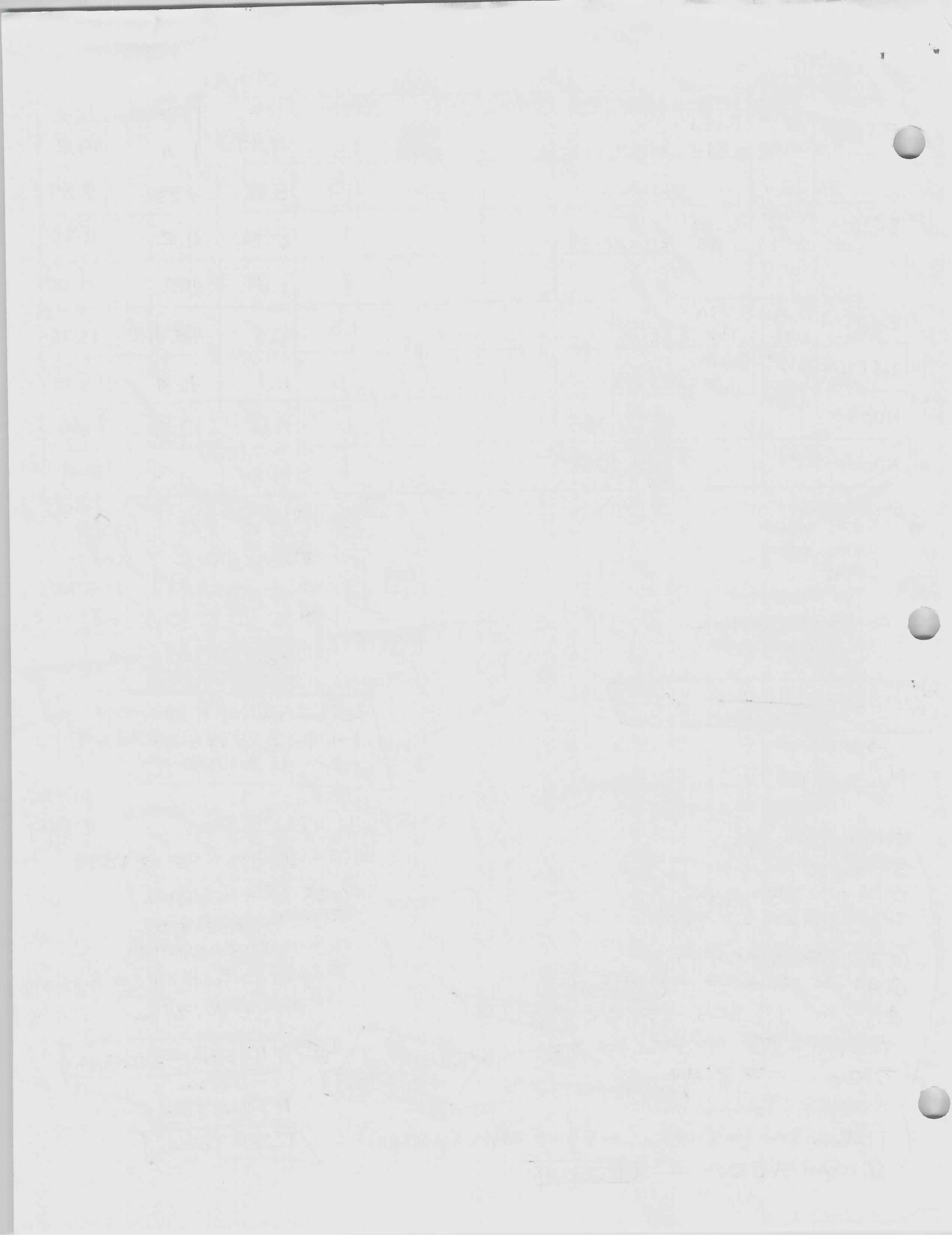
4:05 (11:15 pm → 11:55 pm)

Intra

11:57 pm Final wash

12:10 Done

resuspended
SC @ 25µl
Samples Normal



• Machine start before 7pm^{1:40}
Naming: Group: Inf 001
 or NDOSO-v1

QC beads @ 7:55
 Left 2005

PMA -

ctrl - Unstained

Sample: Ctrl - Antibody

- Tetramer

- Tetramer Control

↑ due to shared naming issue

SC's 30 μl: after abort rate stabilized → ~8:15 start,
 (5 sec delay) $\rightarrow 30 \mu\text{l} = 1 \text{ min}$

Σ SC order test AT488 before F.t.c? why?
 recording @ medium (31.5 μl/min)

[No Sample Cell Left Behind]

☛ Check whether CD25 Pe-G5 worked today?

☛ Inconsistent volume, was it the ejact?

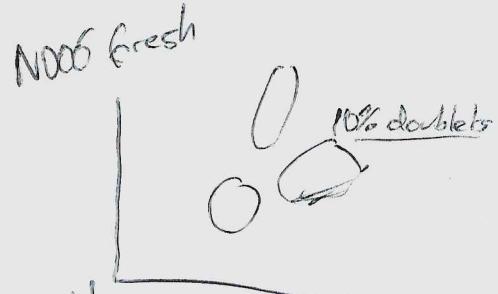
Samples @ 9:19

NDOSO ctrl unstained
 < 65 μl > (~62 μl)
 (~57 μl) → 5900 events /sec abort rate 1.25

NDOSO 13000 event 630 abort 30 μl

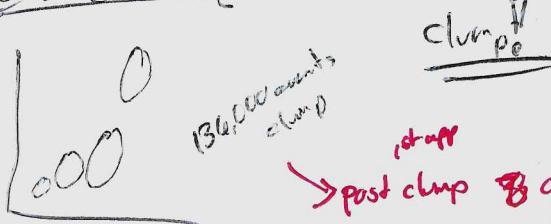
< 1.7M events > (cut to 60 μl → t = 1:58 min)

8300 event 275 abort



(Cut NDOSO PMA final side!)

Counts @ 9:56



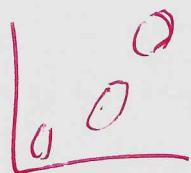
Inf 182

	1st app	2nd app	post clump	30s	counts
tet	16,000	1200	2.2		1.5 M
	11,000	400			2.2 M
					2.3 M
					2.6 M

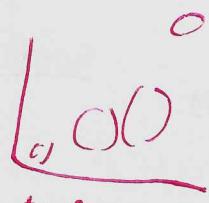
16,800 1300 30 μl

19,000 1500 70 μl
 cut off 10,000 400

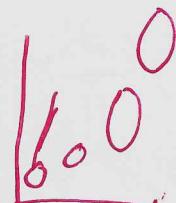
Acquisition continued...'



Inf281
10,500 40°carts



Inf052
20000 1800



Inf171

↑ 14,000 about 1000

? clear cols?

(Done @ 11:19 pm)

Slides @ 11:27 pm

[16 GB data]

	ND050	6	5.5	5	4.5	4	3.5	3
	ND006	6	5.5	5	4.5	4	3.5	3

$$4 \times 3 = \frac{1200}{\frac{\kappa^2}{281}} \quad \kappa_{CO} \quad 3.32 \text{ in } 200\mu\text{l}$$

3.32 in
200µl

Sample	Total	Volume	Concentration	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M	1.50M	
INF182	21.79	4	3	7.26	0.41	0.38	0.34	0.31	0.28	0.24	0.21	0.17	0.14	0.10	0.07	
INF281	22.2	3	2	11.10	0.27	0.25	0.23	0.20	0.18	0.16	0.14	0.11	0.09	0.07	0.05	
INFO52	15.75	2	1.5	10.50	0.29	0.26	0.24	0.21	0.19	0.17	0.14	0.12	0.10	0.07	0.05	
INF171	15.15	~2	1.5	10.10	0.30	0.27	0.25	0.22	0.20	0.17	0.15	0.12	0.10	0.07	0.05	
ND050	9.62	1	1	9.62	0.31	0.29	0.26	0.23	0.21	0.18	0.16	0.13	0.10	0.08	0.05	
ND006	8.24	1	1	8.24	0.36	0.33	0.30	0.27	0.24	0.21	0.18	0.15	0.12	0.09	0.06	
				Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M	
				INF182	0.59	0.537	0.489	0.44	0.391	0.342	0.29	0.244	0.195	0.15	0.098	
R10				INF281	0.73	0.668	0.608	0.55	0.486	0.425	0.36	0.303	0.243	0.18	0.122	
				INFO52	0.71	0.654	0.595	0.54	0.476	0.416	0.36	0.297	0.238	0.18	0.119	
				INF171	0.70	0.644	0.585	0.53	0.468	0.410	0.35	0.292	0.234	0.18	0.117	
				ND050	0.69	0.630	0.573	0.52	0.458	0.401	0.34	0.286	0.229	0.17	0.115	
				ND006	0.64	0.582	0.530	0.48	0.423	0.371	0.32	0.264	0.212	0.16	0.106	
				Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M	
				INF182	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33	
PMA				INF281	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33	
				INFO52	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33	
				INF171	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33	
				ND050	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33	
				ND006	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33	
				Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M	
				INF182	6	5.5	5	4.5	4	3.5	3					
CD107a				INF281	6	5.5	5	4.5	4	3.5	3					
				INFO52	6	5.5	5	4.5	4	3.5	3					
				INF171	6	5.5	5	4.5	4	3.5	3					

1.122

2.241

3.75 mls

38mls

12.8

$$\begin{array}{r} 35 \sqrt{450} \\ 100 \\ \hline 70 \\ \hline 30 \end{array}$$

40

2 2

2 2 2 2

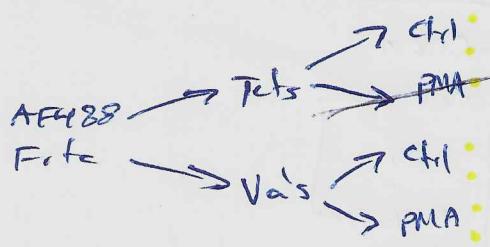
2 2

2 2
2 2

xing #1:

MT unstained for all specimens.
deleted NG0505 unstained to see screen
adjusted negative universal for exp.
using card zombie NFR

Setup to run x screen, duplicate with data



99

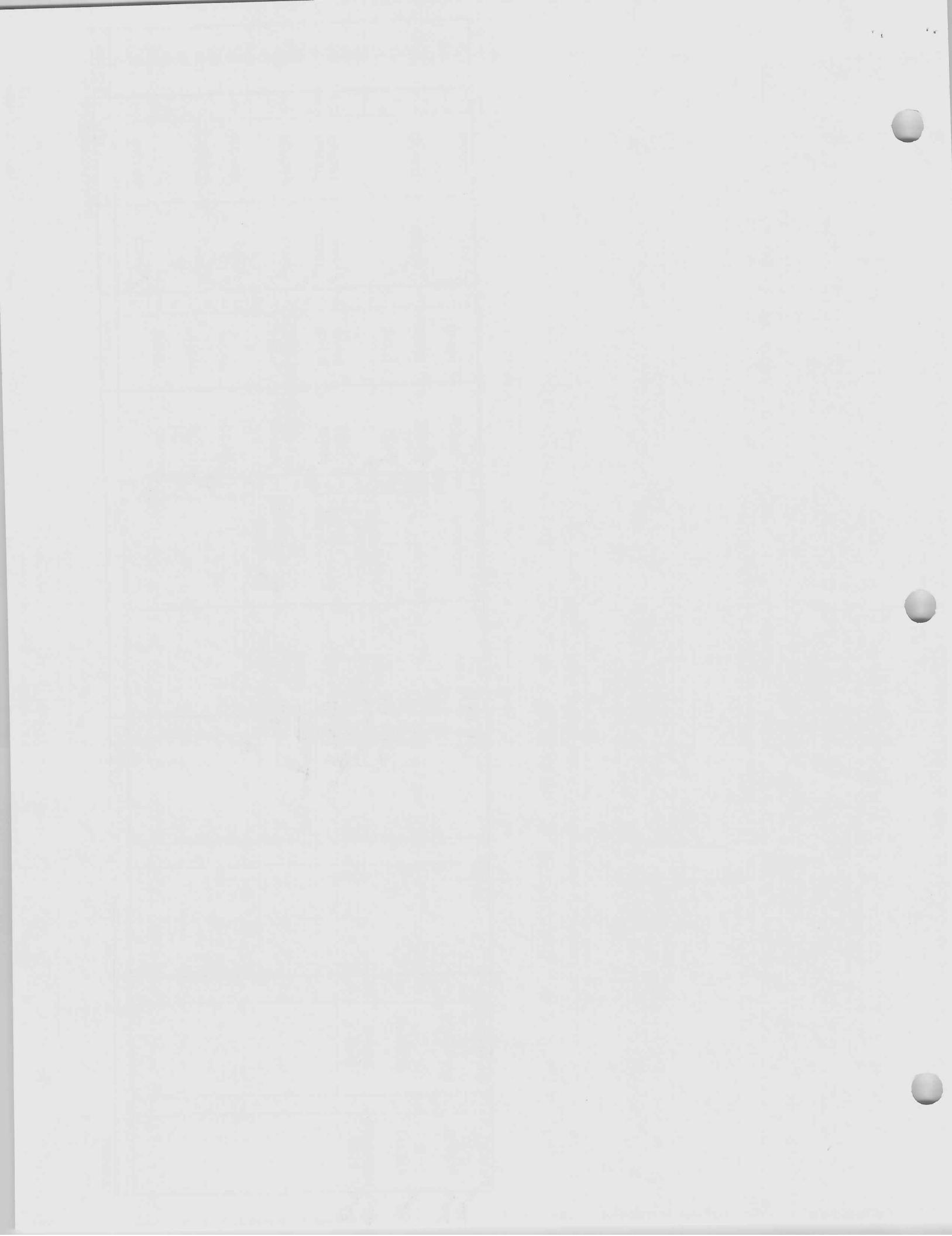
—
B1

9 - 2

ILT & NK SFC Panel

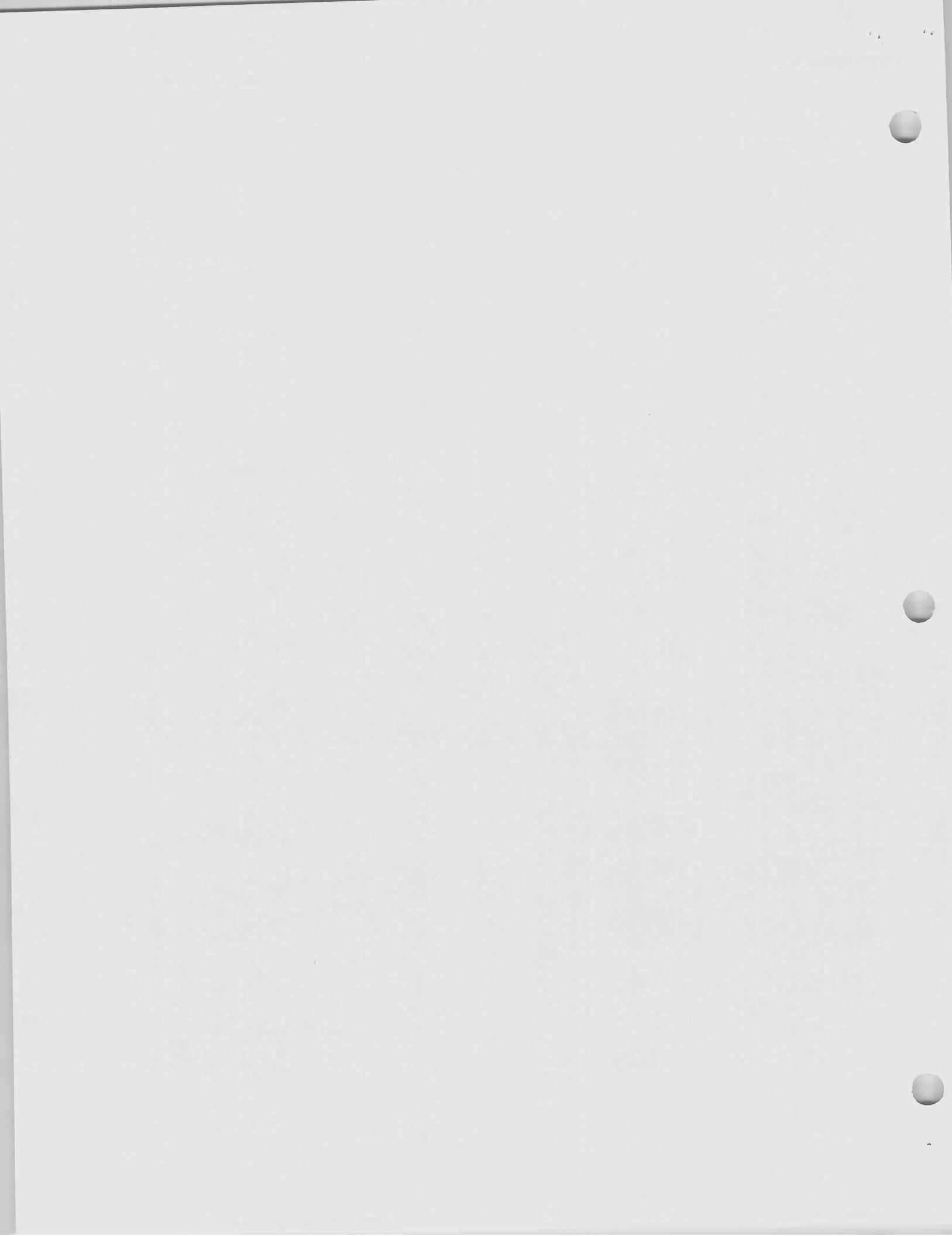
5/12/2023

Spectrum	UV	Violet	Blue	Yellow-Green	Red
373	UV1				
388	UV2	CDUV395	[2] CD62L _{10⁵}		
428	UV3		V1 BV421		
443	UV4		V2 CD127 _{10⁵}		
458	UV5	AF	V3 PacBlue	[1] CD14/19 _{10⁵}	
473	UV6		V4 BV480	[3] CD16 _{10⁵}	
508			V5 B1		
514	UV7	BUV496	[2] CD8 _{10⁵}		
525			V6 BV510		
542	UV8		V7 BV570		
582	UV9	BUV563	[3] CD69 _{10⁵}		
598	UV10		V8 CD45RA _{10⁵}		
613	UV11	BUV615	V9 BV605		
664		BUV661	[3] CCR4 _{10⁵}		
679			V10 BV650		
697	UV12		[3] CCR7 _{10⁵}		
717	UV13		V11 PE-Dazzle594		
733			B7 TNF α		
750	UV14	BUV737	V12 PE-Cy5		
760			B9 PerCP-Cy5.5		
783	UV15		V13 CD7 _{10⁵}		
812	UV16	BUV805	V14 CD7 _{10⁵}		
			V15 CXCR3 _{10⁴}		
			V16 BV786		
			[3] IFN γ _{10⁵}		
			V17 CD7 _{10⁵}		
			V18 CD7 _{10⁵}		
			V19 PD1 _{10⁵}		
			V20 CD4 _{10⁵}		
			V21 V16		
			V22 V15		
			V23 V14		
			V24 V13		
			V25 V12		
			V26 V11		
			V27 V10		
			V28 V9		
			V29 V8		
			V30 V7		
			V31 V6		
			V32 V5		
			V33 V4		
			V34 V3		
			V35 V2		
			V36 V1		
			V37 BV570		
			V38 BV510		
			V39 CD45RA		
			V40 BV650		
			V41 CCR4		
			V42 BV605		
			V43 CCR7		
			V44 PE-Cy5		
			V45 PerCP-Cy5.5		
			V46 CD7		
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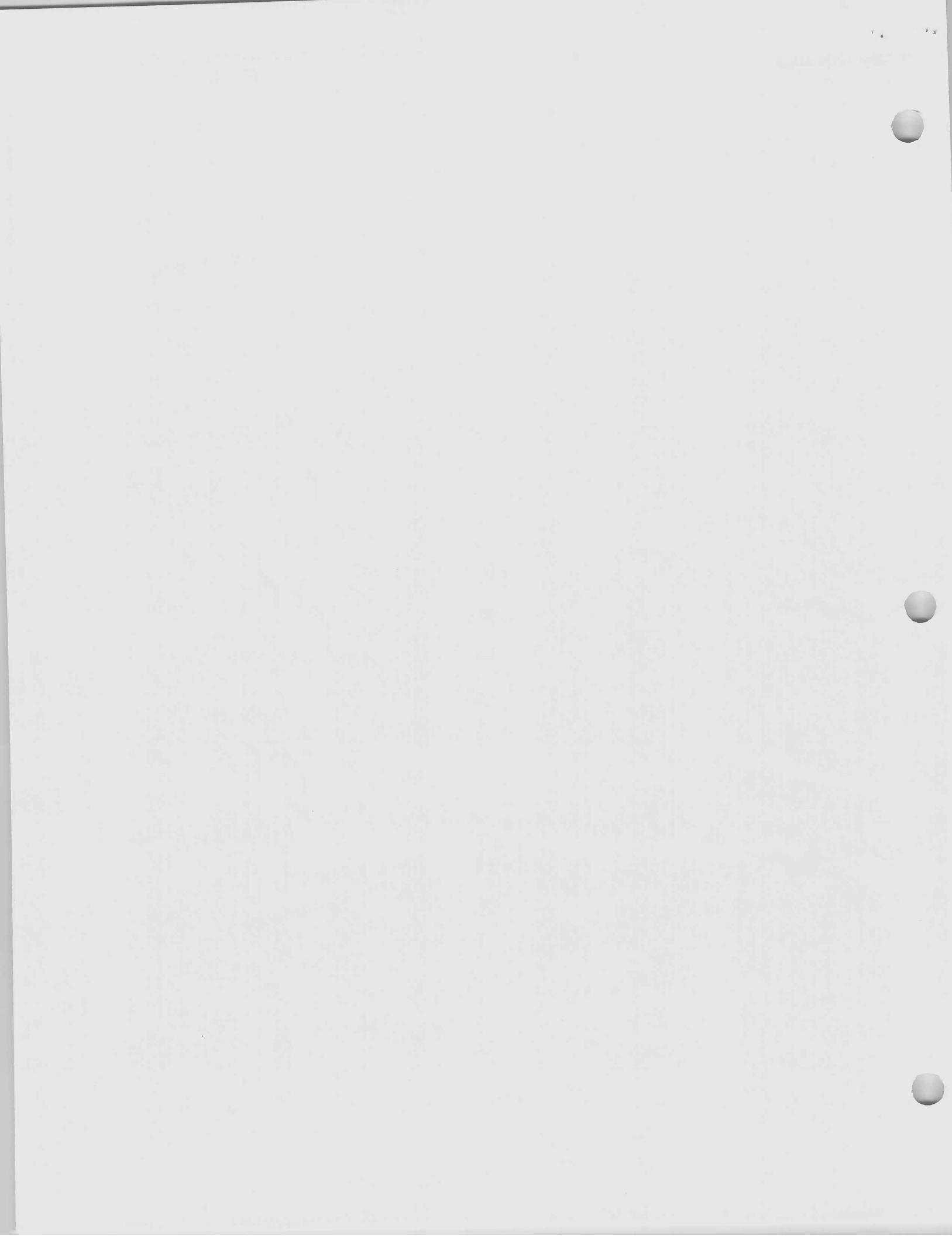
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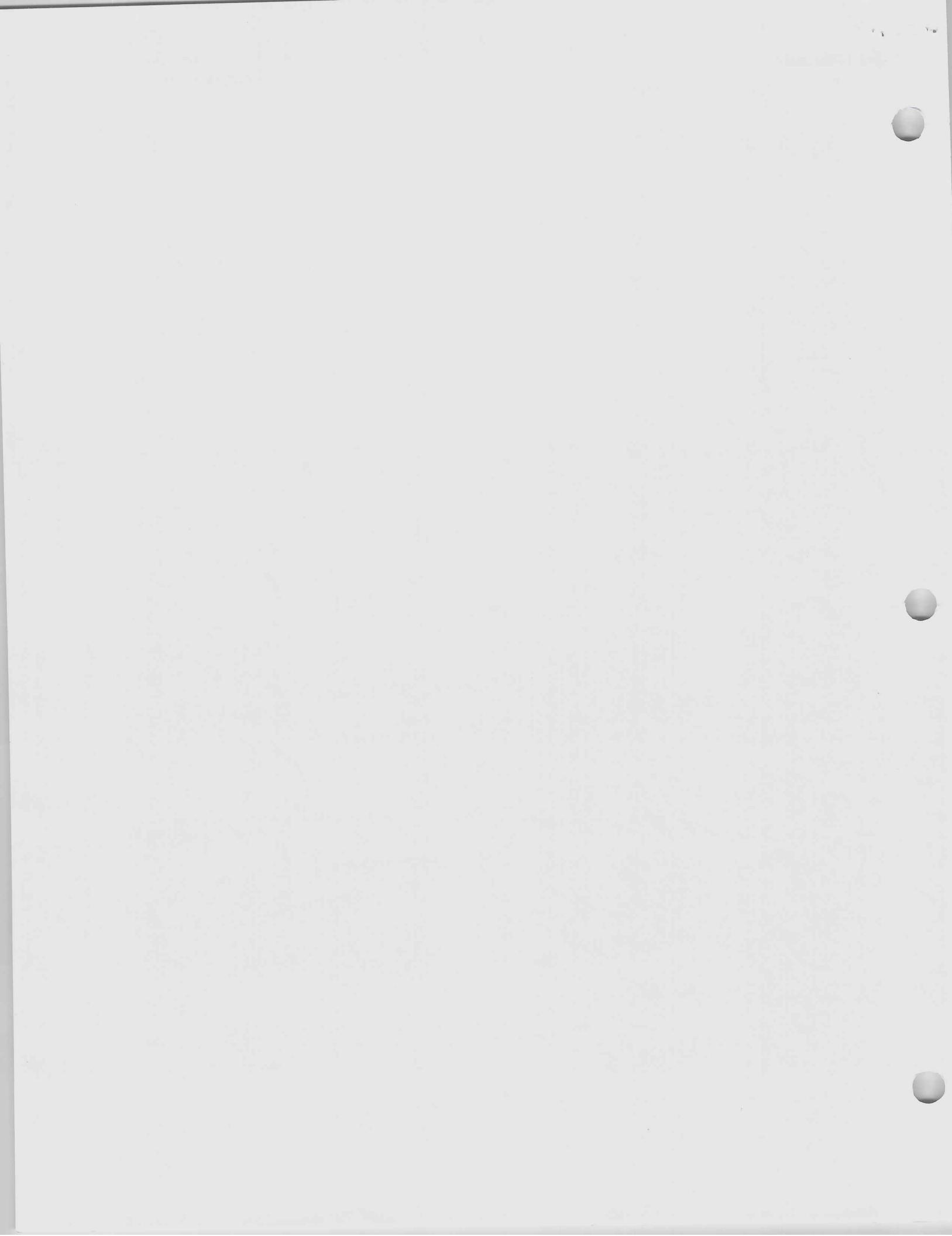
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May 12th, 2023

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