

April 24th, 2023

New Tetramers, New TNFa

NY062 Spm @ 11:12 am
Gordian @ 11:13 am
six count + 11.14 am
count @ 12:02 pm

	con	58ctd	unst	
Inf	ctrl O	0.00	0	ctrl 0.33
	PAK O	0.00	0	PAK 0.33

Rec @ 1:32 pm → 7:32 pm
Abs @ 6:02 pm

Add V_{ext}

Tetrahedron

$\text{OK } \gamma_{\text{inf}}^{\text{ext}}$

$\text{O } \gamma_{\text{ext}}$

$\text{O } \gamma_{\text{ext}}$

$\text{O } \gamma_{\text{ext}}$

Brckl

$\times \frac{1.32}{15.84}$

$\frac{5 \text{ pm}^3}{6.64 \text{ fm}^3}$

$\frac{1.32}{6.64}$

$\frac{2 \text{ fm}^3}{2.64 \text{ fm}^3}$

$\frac{3 \text{ fm}^3}{1 \text{ fm}^3}$

Spin down @ 7:50 pm

8:24pm L/D Zambales Agua/NFC → 8:39 pm
spin @ 8:46pm

63/53

G:07 pm 37°C samples →
Not spin @ 9:41 pm

10:30 jets \rightarrow 40 pm
10:32 vars \rightarrow 32 pm] spin @ 10:38 pm

57.5 55.4

10:54pm 4% for all samples
+ CO₂ spike for POC taken
→ 24pm for spectral
11:10pm for core

$$\begin{array}{r} 14 \\ \times 8 \\ \hline 11200 \end{array}$$

$$\begin{array}{r}
 \times 10 \\
 \cancel{\times 0} \\
 \hline
 11.2
 \end{array}
 \quad
 \begin{array}{r}
 3 \\
 \cancel{\times 0} \\
 \hline
 2.4
 \end{array}$$

RBC lysis 11:30pm (all quoted scis)
Spin @ 11:37pm

17 cold
10 hot
6 cool
8 PMA

29 cold ✓
37 hot ✓

Fix Perm @ 00:13 → 23 → 33 ✓

1st perm wash @ 12:41 pm

2nd @ 12:55 pm

Sc's FixPerm @ 1:00 → 1:10 - 1:20 am

TNFa 1.0 CF 1.2 Dazzle 3.0 ahead for PerCP

PerCP dose @ 1.0 fl

Intra @ 1:27 pm (everything added correctly)

Sc's 1st perm @ 1:32 pm

Sc's in 30 fl @ 2:06 am

2:12 pm Final spin

Done @ 2:32 am

No violent chg! ↗

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Template setup @ 2:24pm!!

or start @ 2:28pm

100K cells

cord: A lot of monocytes in this specimen!!

CD69 - fix ... might be fix. or overstand?

No signal on CD4 BV805

CD127 BV421 ...

double stained?

We are missing the rise in cytokines ↙ %↑ when left in fridge extra time

rename Uazygj18 → AF488 CD3

Export fcs file since its on F.I.C.

the separate stain for CD27, NKG2D, CD69 ≠ work.

TNF α OF594 very bright

got sniped in acquisition, flip.

TNF α Dazzle

PcG-5? might be missing? looks BVV-ish?

1/1 CD26 PerCPSSS5 ≠ work

CD107a spectral

Δ in cell surface after fridge?

Sc's done @ 3:28pm

CD107a PMA → PerCP Cy5 sample

INF426 unstained PMA appears to have signal ooo

Screened up unmixing on cow background (not detecting sample wst)

no CD4 ab in CD4 tube

the intraspillle show some tailing

New donor
0.14%

0.02%

<3%¹⁷

CD25? CD4? CD127?

Unmixing using both TNFas.

more PerCPy5.5

© 1.0 still nearly off chart

CD26

PE-CF594

10⁴/₂-10⁵/₂

peak @ 10⁴

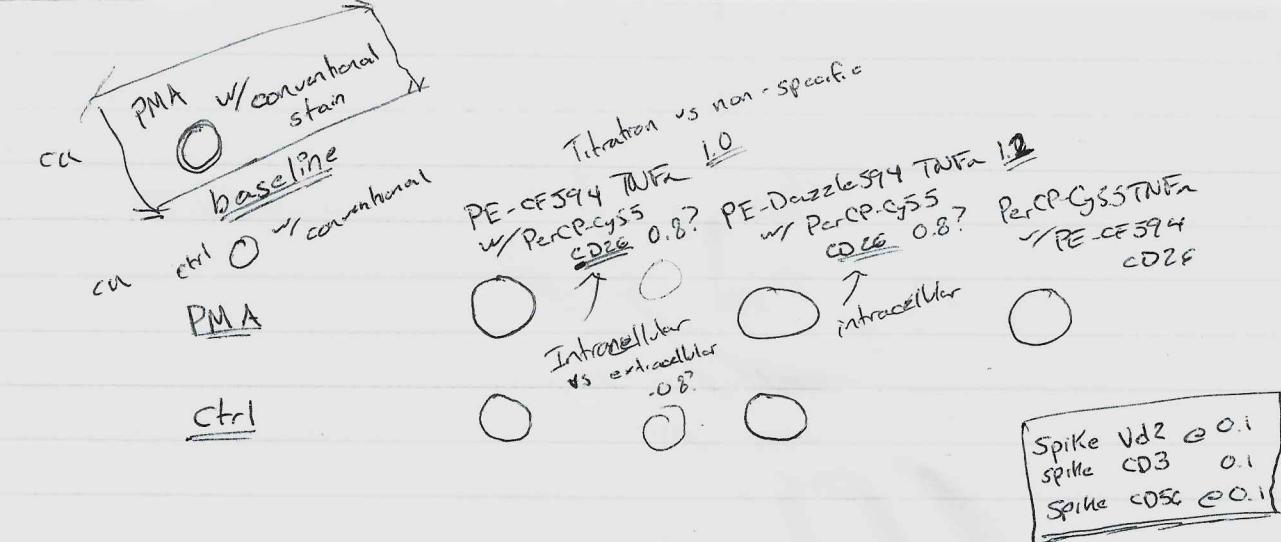
10⁵-10⁵/₂

TNF α

10⁶ TNF α

below 10⁴, work?

Dazzle 10⁵/₂ - 10⁶/₂



Ver 2.2 chris

New test: OP-RU 0.5 0.3 0.1
 G-FP 0.5 0.3 0.1

? as whole panel?
waste of antibodies

New SC set

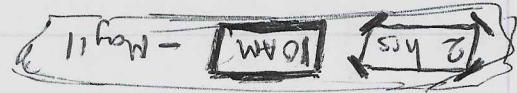
+ bead controls for D17 → permwash
→ nuclear wash

+ scs for tets (biotin)

+ SC's for TNF_αs

Inf212 6 card 2019

female	394-4	9 2 2	Box 12A	Row E	col 5
male	426-2	12 2 2	Box 12A	F	col 1
Male	414-9	13 0 2	Box 12A	Row H.	col 8
female	442-0	11 2 2	Box 13A	Row A,	col 9



CuO

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
INF426	18.95	3	6.32	0.47	0.44	0.40	0.36	0.32	0.28	0.24	0.20	0.16	0.12	0.08
NY062	24.52	3	8.17	0.37	0.34	0.31	0.28	0.24	0.21	0.18	0.15	0.12	0.09	0.06
NA	5	1	5.00	0.60	0.55	0.50	0.45	0.40	0.35	0.30	0.25	0.20	0.15	0.10
NA	5	1	5.00	0.60	0.55	0.50	0.45	0.40	0.35	0.30	0.25	0.20	0.15	0.10
NA	5	1	5.00	0.60	0.55	0.50	0.45	0.40	0.35	0.30	0.25	0.20	0.15	0.10

Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
INF426	0.53	0.481	0.437	0.39	0.349	0.306	0.26	0.218	0.175	0.13	0.088
NY062	0.63	0.580	0.527	0.47	0.421	0.369	0.32	0.263	0.211	0.16	0.106
R10	NA	0.40	0.366	0.333	0.30	0.266	0.233	0.20	0.166	0.133	0.10
NA	0.40	0.366	0.333	0.30	0.266	0.233	0.20	0.166	0.133	0.10	0.067
NA	0.40	0.366	0.333	0.30	0.266	0.233	0.20	0.166	0.133	0.10	0.067

Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
INF426	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
NY062	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
PMA	NA	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5
NA	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
NA	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
NA	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
INF426	6	5.5	5	4.5	4	3.5	3
NY062	6	5.5	5	4.5	4	3.5	3
CD107a	NA	6	5.5	5	4.5	4	3.5
NA	6	5.5	5	4.5	4	3.5	3
NA	6	5.5	5	4.5	4	3.5	3

34

Ex-vivo gd-CK: PMA-ionomycin									
#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	2	Intracellular 40min @RT	2
1	V450	BV421	PD1			2	4		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD27			1.5	3		
4	B530	Alexa 488	Vδ2			1.2	2.4		
5	B710	PerCPeF710	CD3			1	2		
6	Y590	PE	CD56			0.5	1		
	Y615	PE-Cy7							
7	Y780	PE-Vio770	IFNγ					0.6	1.2
8	R670	Alexa 647	TNFα					2	4
9	R780	APC-Fire750	CD45RO			2	4		
Antibody Total					8.2	16.4	2.6		5.2
PBS					12.3	24.6	17.9		35.8
Pipette draw volume / sample					19.5		19.5		

737

421

Surprisingly clear

PMA CD3 - what off? CESM
dimp

Conv PMA PE - PerCP stripe unmovey

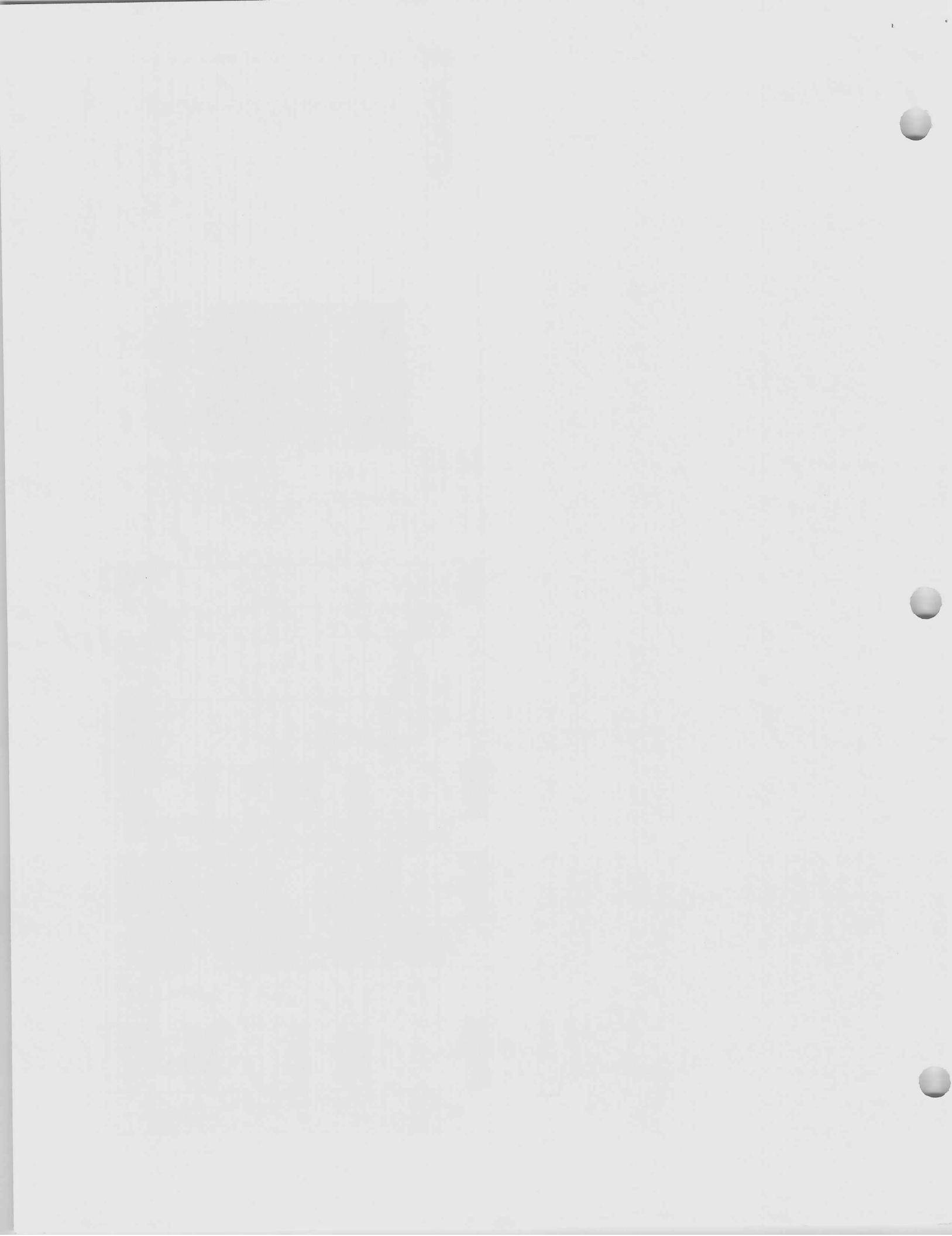
Unloaded 1
original large

▼ Tufts are moving
not ardent

NKT ab < NKT tet ... fascinating...

< T cell spread >

#	Filter	Single color (μl)	Ref ctrl	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial/Lot #	During stim!!!	8	U/D 15 min (RT)	Tetramer 40 min @ RT	HotStain 30min @37C	7	ColdStain 30min @4C	7	RBC Lyse, then FixPerm	Spiked 30 min @ RT	7
UV2				BUV395	CD62L	(DR5G-56)											1.2	8.4	
1	UV7			BUV496	CD8	(RPA-T8)											0.7	4.9	
2	UV11			BUV661	V62	(B6)											0.7	4.9	
3	UV15			BUV805	CD4	(SK3)											0.1	0.7	
4	V3			Pacific Blue	CD14	(MS12)											0.5	3.5	
V3				Pacific Blue	CD19	(HB19)											2.0	14.0	
5	V5			BUV480	CD161	(REAG31)											2.0	14.0	
6	V7			BUV510	CD45RA	(HI100)											0.7	4.9	
7	V10			BUV605	CD56											1.0	7		
8	V11			BUV550	CCR7											1	7		
9	V13			BUV711	CD7											2	14		
10	B2			AlexaFluor 488	hCD1d											0.7	4.9		
11	B3			Spark blue 550	CD3	(SK2)										< : > (1)			
12	B4			PE	NKG2D											1.2	8.4		
13	R1			APC	CD16											1.5	10.5		
14	R2			AlexaFluor647	V67.2											0.7	4.9		
15	R6			Zombie NIR	L/D											<1:2500>	< : > (1.5)		
Antibody Total								0.0	0		Antibody Total	8.0	56	10.4	72.8		2.2	15.4	
And UNSTAINED CONTROLS !!																			
R10 Media				20.5	164	Brilliant Stain													
Pipette draw volume /sample				19.5		Pipette draw volume /sample													
								55											



Wash with 2 ml PBS, spin down 1300 rpm, 8 min
 800 uL of Liebermann mix, 1 (2200 uL) @ RT for 15 min
 Wash 2 ml 5% PBS-FBS, spin 300 rpm, 8 min

Add HotStain mix, incubate @ 37°C for 30 min
 Wash 2 ml 5% PBS-FBS 1400 rpm, 8 min

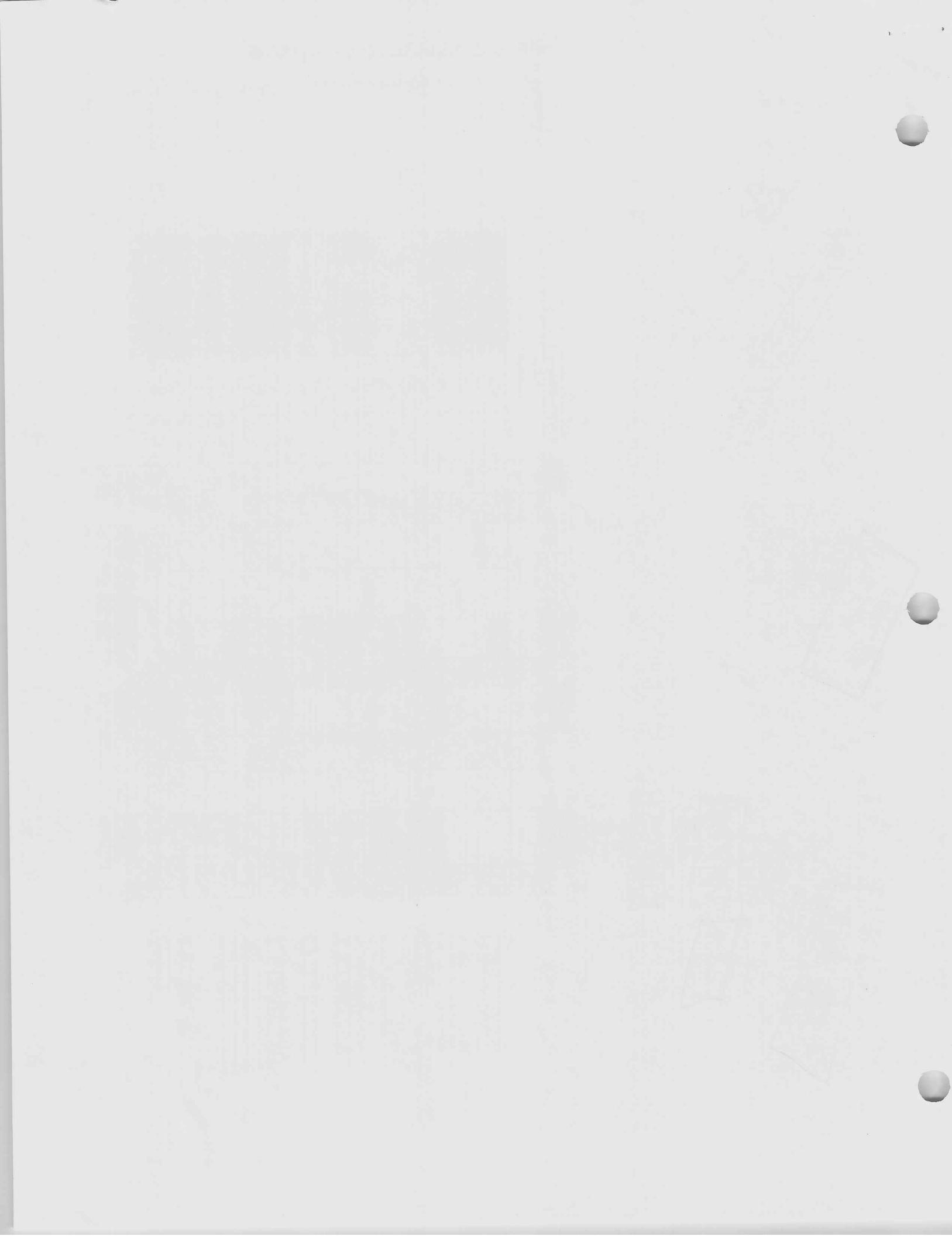
Add EtOH, incubate @ RT for 10 min
 Wash 2 ml 5% PBS-FBS 1400 rpm, 6 min

Add ColdStain mix, incubate @ 4°C for 30 min
 Add 300-500 uL 1X RBC Lysis for 3 minutes
 Wash 2 ml 5% PBS-FBS 1400 rpm, 6 min

300 uL BD FixPerm, incubate @ 4°C for 20 min
 (vortex every 10 minutes)

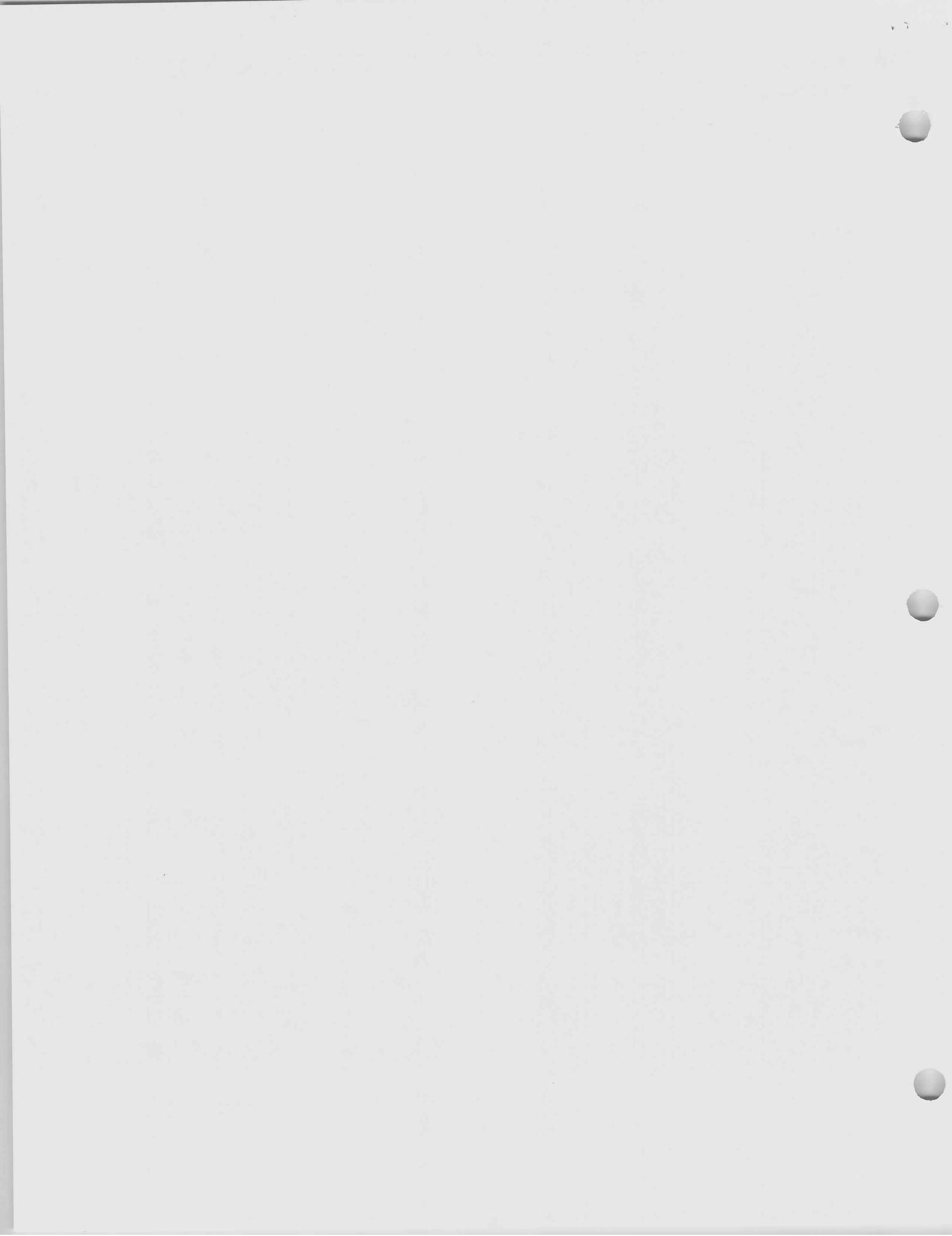
First Perm/Wash:
 Second Perm Wash:
 1 ml Perm/Wash 1500 rpm 6 min
 Add Intracellular Stain, incubate @ RT for 40 min
First Perm/Wash:
 2 ml Perm/Wash 1500 rpm 6 min

Resuspend in 70 uL 0.4% PFA-BSA



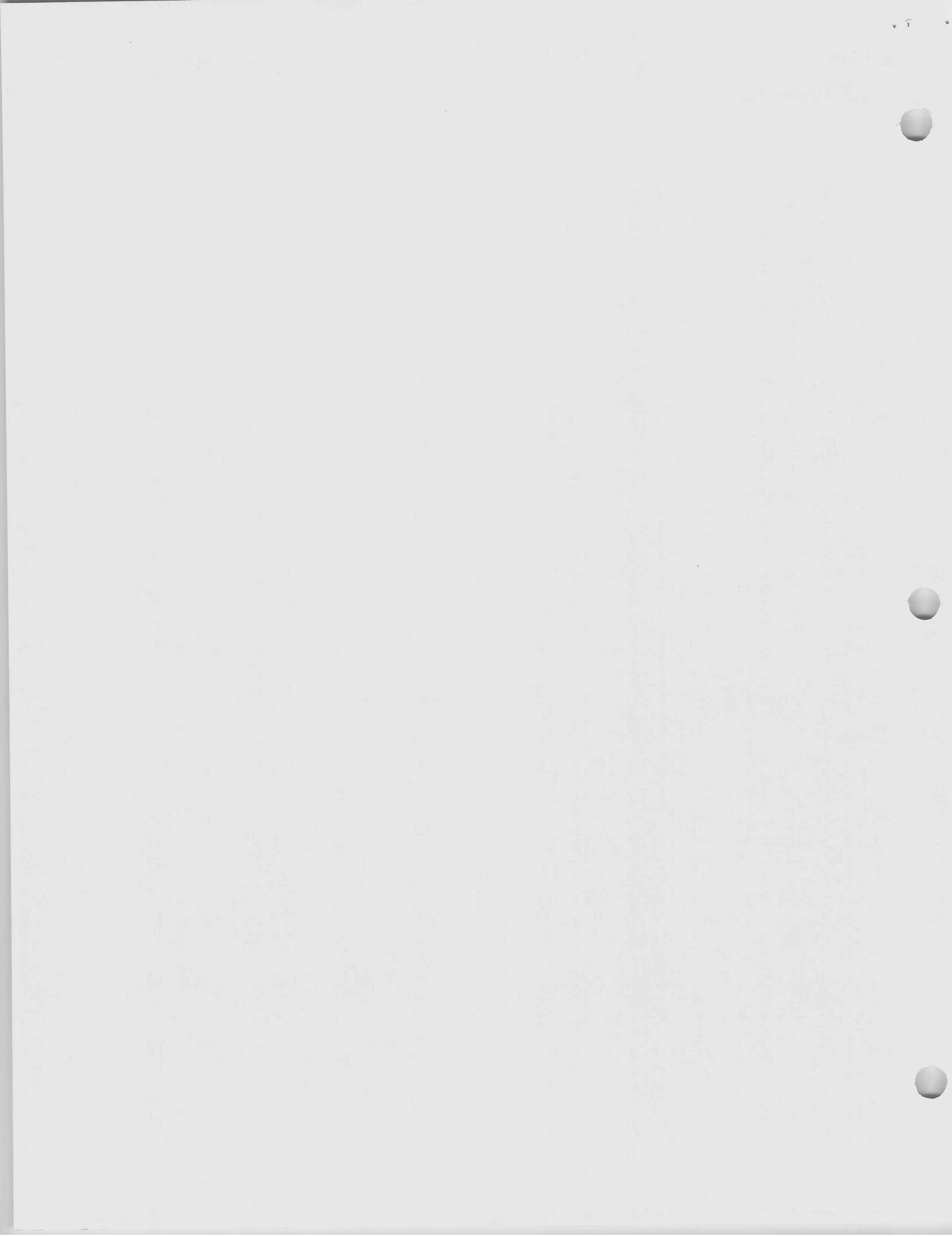
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