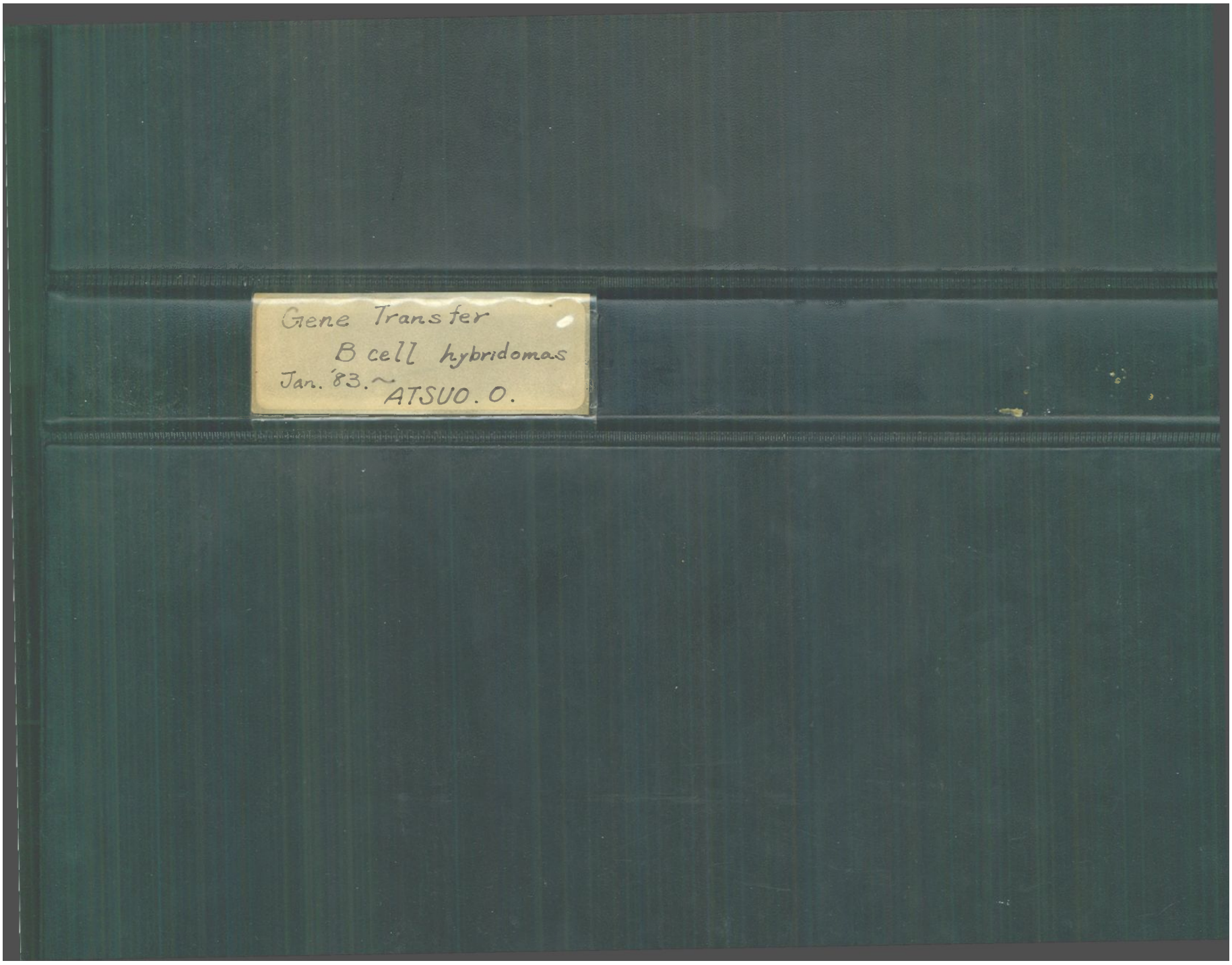

~~Result of antibody titer~~

Antibody activity of Transformant culture soup:

Recipient 208 Vector H-L-~~sep~~pSV2neo

89 clones were ~~so~~ surnaied. 1:1 1:10

ELISA overnight

		1:1	1:10			1:1	1:10		
1	2			31	36			61	(80)	+	+	(-)
2	3	±	-	32	37			62	82			
3	4			33	39△	±	±	63	(87)	±	+	
4	5			34	40			64	(89)	-	±	
5	6			35	41			65	(91)	-	+	
6	7			36	42			66	92			
7	8			37	43			67	(93)	-	±	
8	9			38	44			68	95			
9	10			39	45			69	96			
10	11			40	46			70	97			
11	12			41	47			71	98			
12	14△	±	±	42	48			72	99			
13	15			43	49			73	100			
14	16			44	50	±	-	74	101			
15	17			45	51			75	102			
16	18			46	55			76	104			
17	19			47	58			77	105△	+	±	
18	20			48	60			78	106			
19	23			49	61			79	107			
20	24			50	62			80	108△	±	±	
21	26			51	63			81	109			
22	27			52	64			82	110			
23	28	±	-	53	68			83	112			
24	29			54	70△	±	+	84	113			
25	30			55	71			85	114			
26	31			56	72△	+	±	86	116			
27	32			57	73			87	117			
28	33			58	74			88	119			
29	34			59	75			89	120			
30	35			60	78							

T U

10

Haemagglutination titer about the final clones
Jan. 13 83

Sp	10240	
R8	20	
9	<1/2	
12 11	32	10d
19	5120	(5120)
22	640	
31	320	
T1	<1/2	
3	~~1/16~~	
T12	<1/2	
T17	~~1/16~~	
014	<1/2	

Transient expression about Ig M-10-1

Hybridized 14. Jan. 83

No. 1 ~ 32.

2 ~~4~~ 5, ~~8~~ 12, 17, 20 21, 29 (+)

T1 T12 T3 T1Z R9 R11 R80 R81 R22 R19 SP
L2 e L2 L1 L1 L1 L3 L1 L4 L1 L4 603
C14
Exposure. O/N.
Jan. 19. 1983.
KODAK SAFETY FILM ARO
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KODAK S

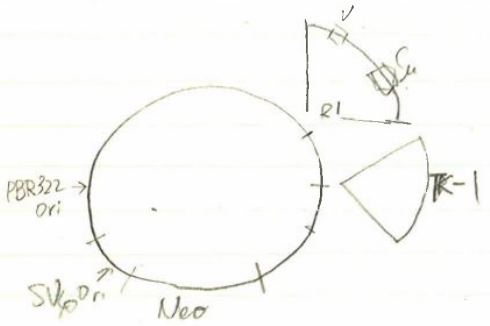
Recipient Cells:

X-63 208 Sp2/0. IgM-10-1

Heavy chain probe:

total 15.4 kb

partial digest and ligate to pSV2neo-TK1 pR-TK1



Heavy chain - κ chimeric Vector

anti-TNP antibody activity. 20. Jan. 83

Samples Sp 2/0 : K_{pc}. H_{pc} less

Vector ⓐ TNP_{Heavy} - TNP_{lc} - pSV2neo

Well No 1~30 - Atsuo plate 96well
31~43 - Telessa plate 96 well
44~45 - テルサ 24well
46. small flask

Control (−) Supernatant from Transformant(−) wells

(+) Sp603

by ELISA ⊕
24, 30, 38, 40. 41, 42, 45. 46 ~~not~~

~~8?~~ 8. 11

Confirmation experiment. 22~23 Jan 83

No		
24, 30. 46.	24, 30. 46	+
		−

EcoRI.: pSV2neo

T. pRTK1 100 pg

c. f. 18h.
rest. 48h.

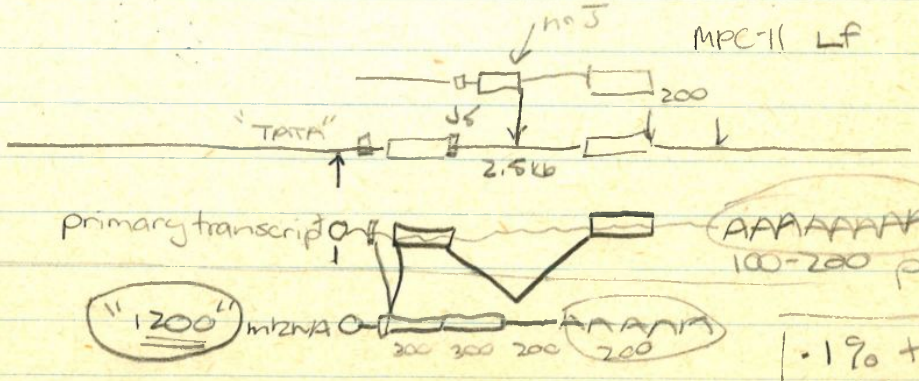
R. e 2.5h.
rest 18h.

Hirt. 1x10⁶ cells

wt 3. 4 μg
wt 2 1 μg
wt 1 0.4 μg

Baltimore

1.2
1.9
2.6



pdsA 1-3% / cytoplasmic > 1000

·1% total cytoplasmic

Jan 28. 83

Limiting Dilution

Sp 2/0 1, 4, 8.

Results of 1 Selection

R11

1mg		~~1/64 2/64 64~~	~~2/64~~
free	20d		1/64
free	30d		1/64
5mg	20d	1/32	1/64 ±
	30d		1/64

R19

1mg			1/5200
free	20d		~~1/5200~~
	30d		~~1/5200~~
5mg	20d		~~1/20000~~
	30d		~~1/20000~~

Anti-TNP activity of Culture soup 25. Jan
of. Sp2/0 H-L pSV2neo

40 colonies positive Wells from Teresa

ELISA assay. O/N

1 ~ 40

Positive 1. 6, 9, 10, 32, 40#

bottle ⊖

LPS from ken 5mg/ml

50μg/ml

Jan. 28,

Anti-TNP antibody activity of X-63 or Sp2/0 Culture soup

Cell		
	X63	H-K-pSV2neo
	Sp2/0	H-K-pSV2neo

X63 1 ~ 8 (Teresa) 9. Aswo

Sp2/0 1, 10, 40 Confirmation

X63. 4, 8, + (8 #) 1, 6 ± ?
SP2/0 1, 40, +
Confirmed - !!!

Result of anti-TNP antibody titer check of ~~Sp~~ Ig M-10 and Sp2/0 and X-63 transformant

1 ~ 37 Ig M-10

10, 20, 24, 25, 35, ± or +

38 ~ 79. X-63

43, 46, 50, 52, 57, 67, ~~SR10~~?

SR 10 (-) bottle, , Ig M-10 bottle (-)

Previous experiment

42 well were checked

++	4
+	7
±	2
-	29

7, 10, 16, 19, 23, 24, 27, 33, 36

- ± + ± + + ++ ++ +

36, 37, 40, 41, 43

+ ± + ± ±

Jan. 31.

Samples for Mark

from Teresa (Sp 2/6) 1, 2, 3, 4

7. 10. 16. 19, 23. 24, 27, 30, 33, 36; 37
40, 41, 43.

Limiting dilution

X-63 No 16, 19, 27, 23, 33

Result from Mark. ag's
Radio immuno assay

7, 10, 16, 19, 23, 24, 27, 30
- - + ++ + - ++ -

33, 36; 37, 40 41 43
+ + ± + - -

Sp 2/6 all negative 1. 2, 3. 4

Feb 3

LPS stimulation start. 18:00.

R11 5×10^5/ml

R19 5×10^5/ml

50 μg/ml LPS. DIFCO.

Result of selection on LPS

R19. 1mg.

Feb. 7. '83

Protein Gel

~~[illegible]~~ x63 x63 x63 x63 x63 IgM10 IgM10
~~[illegible]~~ 16 19 27 33 ① ②

T12e R8C2 R9C6 C14 Sp603
new new new new

all 1:1. 12.5 λ + 12.5 λ

Titration of Transformant

1	2	3	4	5	6	7	8	9	10	11	12
1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	5120			

Sup. 25λ. TNP RBC 20λ. 1:10 diluted Rαμ

1	Sp	1/5120
2	C14	< 1/20
3	T1L2	< 1/2
4	T3L2	1/20
5	T12e	1/2
6	T17L1	1/40
7	R8 ~~Q~~L2	1/20
8	R9L6	< 1/2
9	R11L3	1/80
10	R19L1	1/5120
11	R22L6	1/2560
12	R31L4	1/1280
13		

R11. 10 d. all 1:1280

2M3-M8 14/181

204 - 50/212

BW —

9. Feb

R19	1mg	2560	
		~~1/40~~	
free	20d	~~2560~~	~~2560~~ 5120
	30d	~~5120~~	5120
	40d	5120	2560
5mg	20d	~~2560~~	2560
	30d		5120
	40d		2560
LPS	72h		5120

R11	1mg	1/40 ~	1/64
free	20d		1/64
	30d		~~1/64~~
	40d		1/32
5mg	20d		1/32
	30d		1/64
	40d		1/32
LPS	72h		1/64

mic. X X 1/40 1/4

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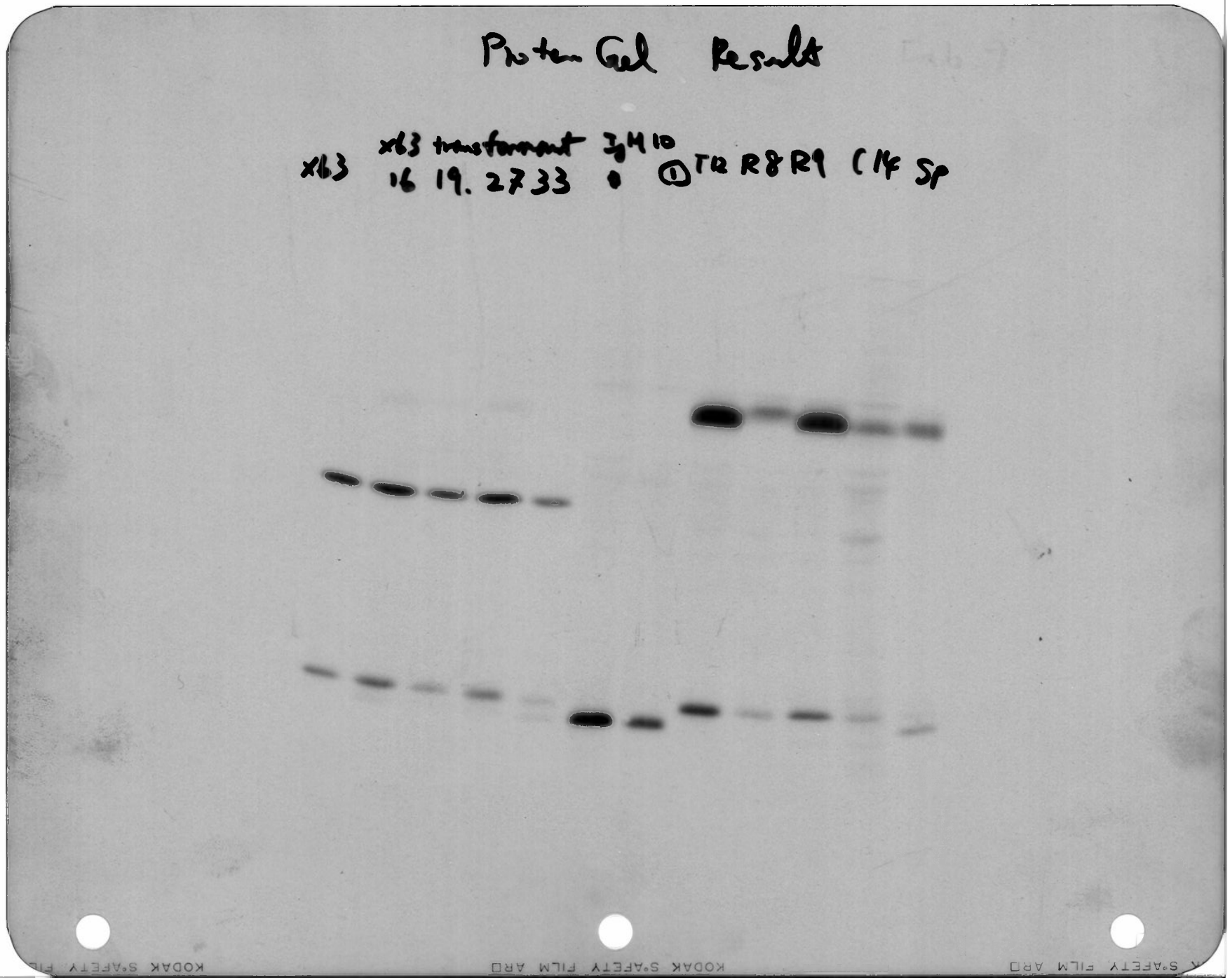
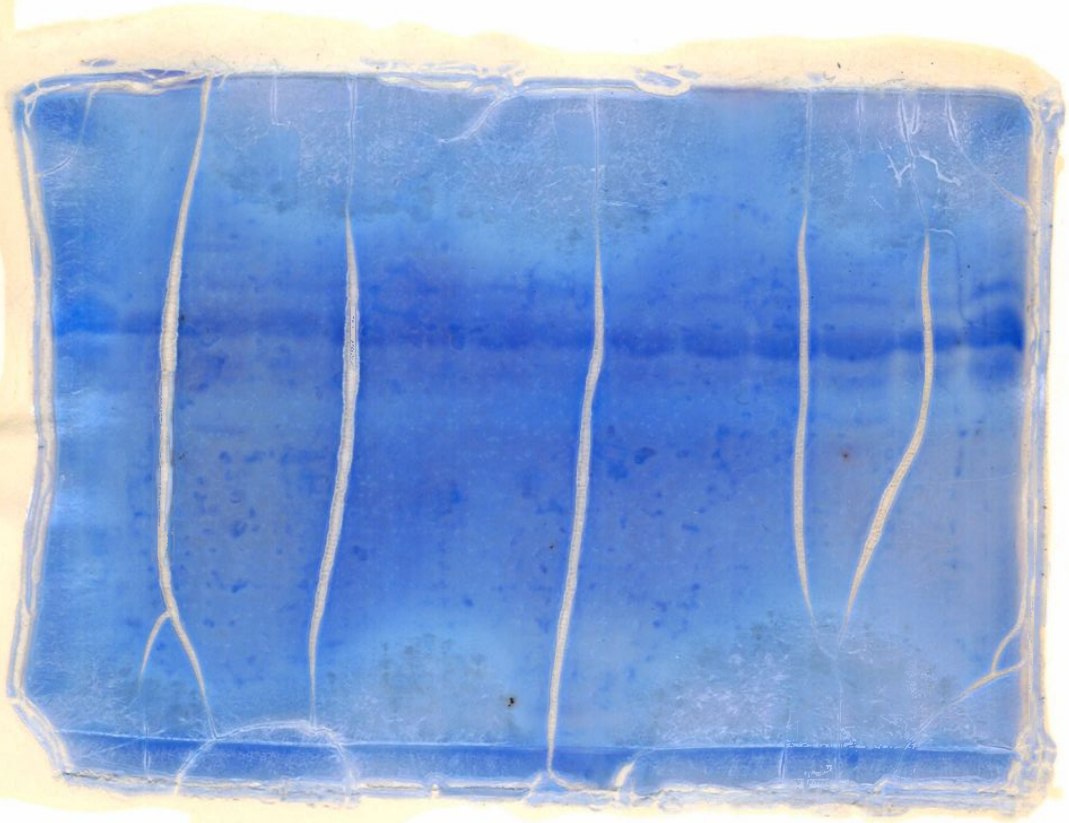
KODAK SAFETY FILM ARO



Feb 7.83



Feb 10 ~ Feb 11

Check of anti-TNP antibody activity of culture-soup from Ig M/D - transformant

Previous data ~ 3/68 (+)

1 ~ 48. from 2 well plate
2, 8, 31, 38, 42 (+)
5, 11, 16, 20, 21, 32, 35, 36, 39, 40, 45 ± may be weakly positive,

49 ~ 69. from 96 well
52, 54, 56, 58, 62 69 (±) color change weak.
59, 68 (+) confirmed.

Limiting dilution
2, 8, 31, 38, 42,

Check of anti-TNP antibody titer Feb 13

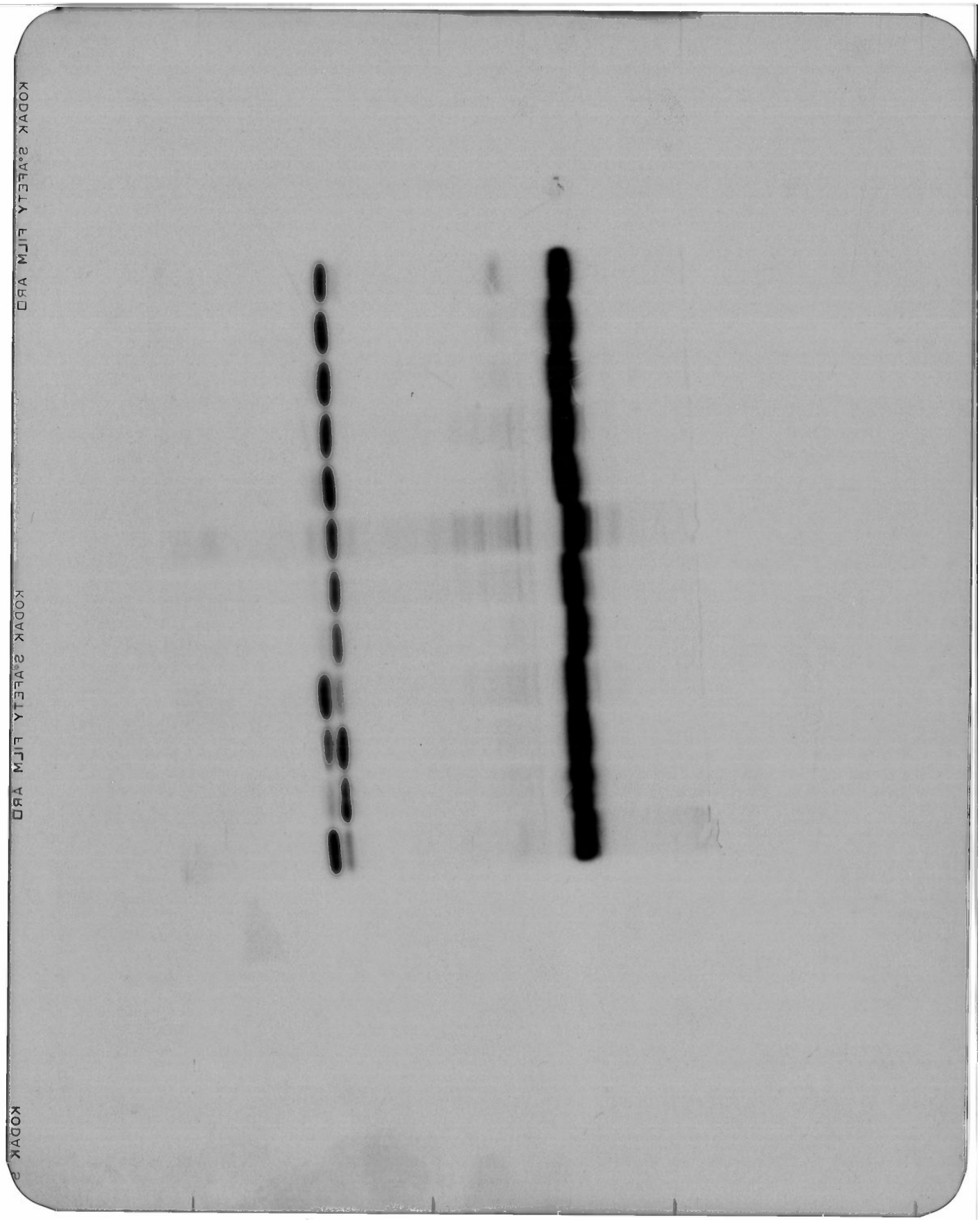
H-neo Sample Ty M-10 transformant from Teresa

H-L neo
- X63-19 done No 4
- -33 done No 1
- Sp2/0 done No 7
- 10

Confirmation

Result X63-19 No 4 Strongly (+) (+) (+) ± → Small flask.
33
Sp2/0 7
10

C14T1 T3 T12 T17 28 29 211 R19 R22 R31 SP.
L2 L2 e L1 L1 L4 L3 L4 L6 L4 603
13.Feb.'83
+8h.
28.9.01.11


KODAK SAFETY FILM ARO
KODAK SAFETY FILM ARO
KODAK SAFETY FILM ARO

1. Mar. 83

Thaw the cells

R. 8. — 6788.
R. 11 6788.
R 19 6787.

C_{14}-Leucine. label. { X63. transformant. 19.
33
Sp 40.

g

Check of anti TNP antibody activity of IgM-10 transformant

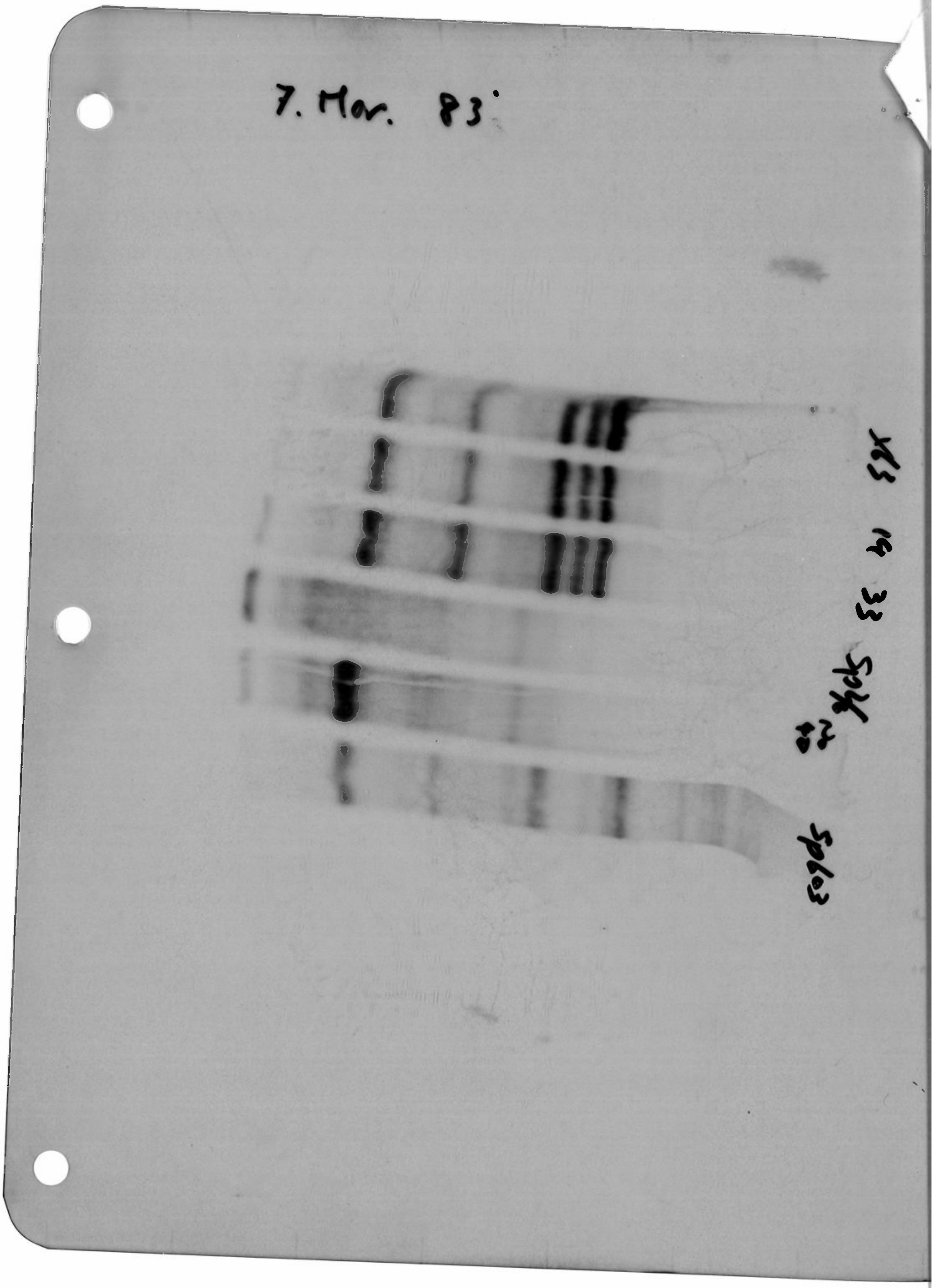
31 24 well plastic dish wells were examined

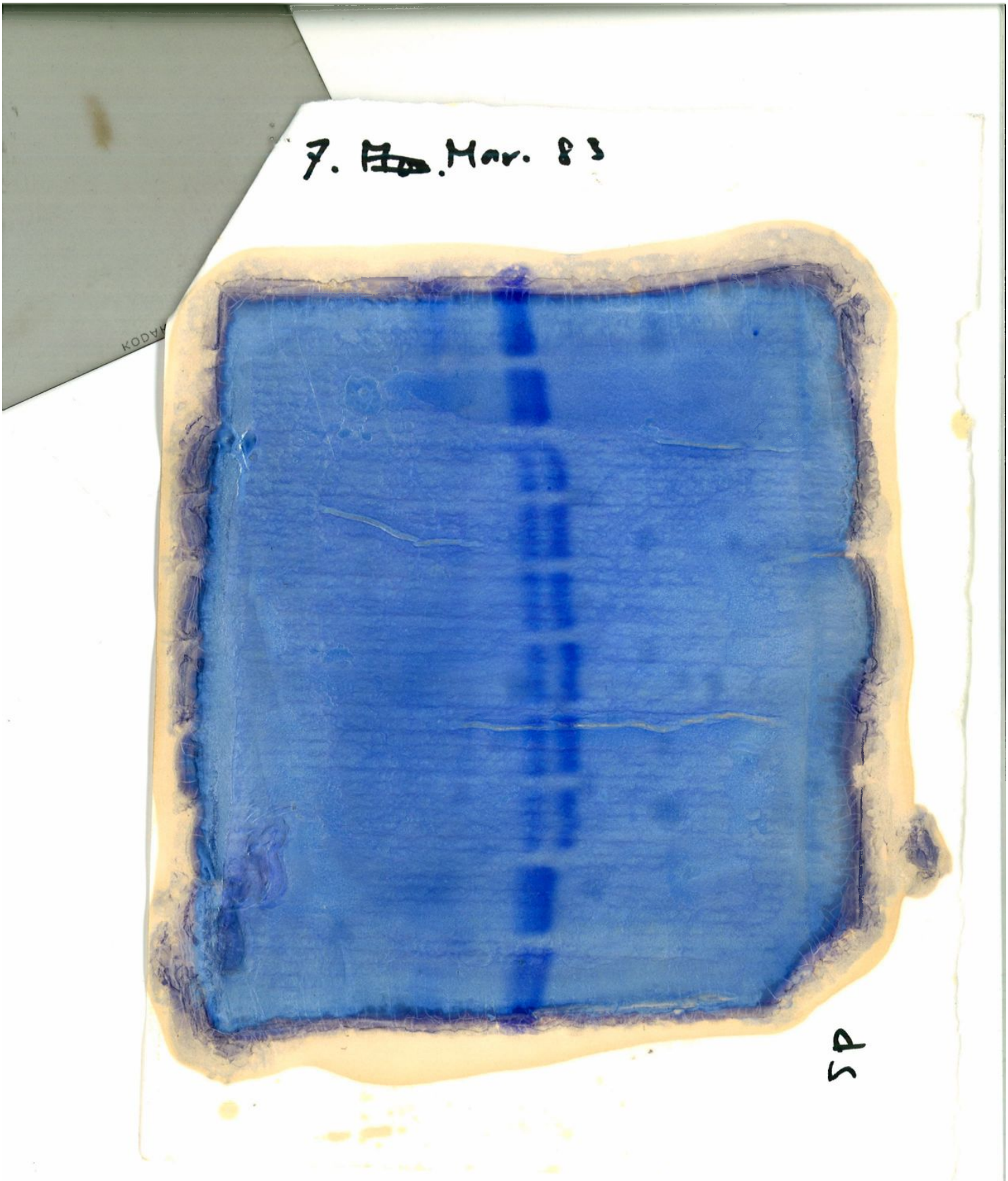
3 wells are + → 2 wells ++
and 1 wells weakly +

1 well bad viability.
1 well No. 88 good viability

1 flask No. 40 ??? negative

7. Mar. 83
KODAK SAFETY FILM ARO


7. Mar. 83
Sp65
14
33
Sp64
Sp63


7. Mar. 83
SP

unreduced

×63 19 ~~3B~~ SR10. SR.40. Sp603

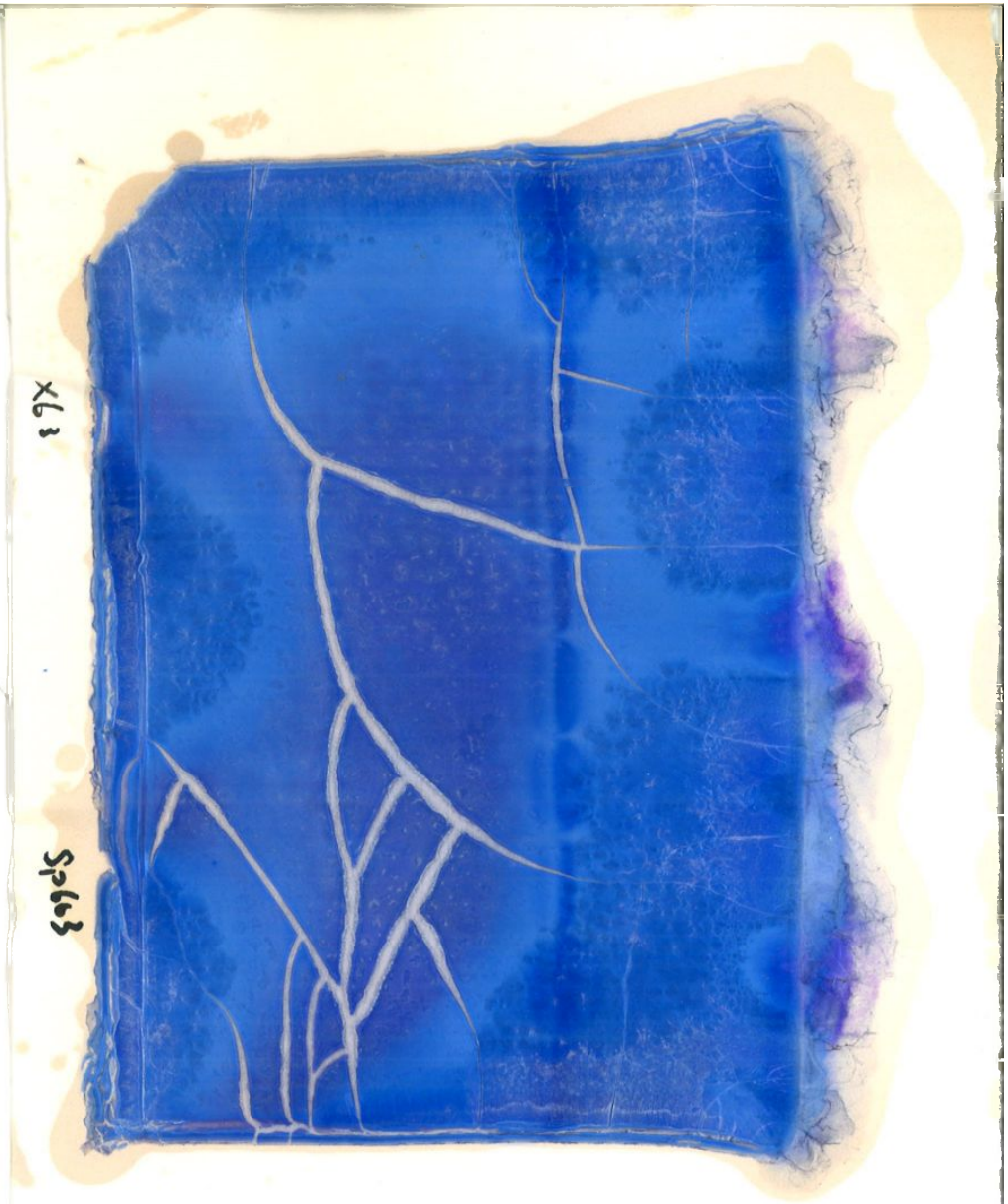
7. Mar. 83

Protein Gel ~~to~~ reduced

×63 19 33 Sp2/0 SR40 3p603

Result of Unreduced Gel

failure
to long time incubation (boiled).



Bottin gel.
9. Mar. 83

Screening of Mar. 10. 83

x NP transformant

72 samples # 2, + 2.

~~14. Mar.~~

~~Enrichof~~ Surface Immunoglobulin + alls

Protein Gel Unreduced 14 Mar. 83'

Protocol

– – x63 x19 x33 Sp3/0 SR40 Sp603 – –

16. Mar 83

Staining of H-k Transformant

Cells

I_g MLD	—
Sp603	—
R44 (I_gMLD transformant)	—
R45 (Sp2/0)	±
x19	++
x33	++
x63	±

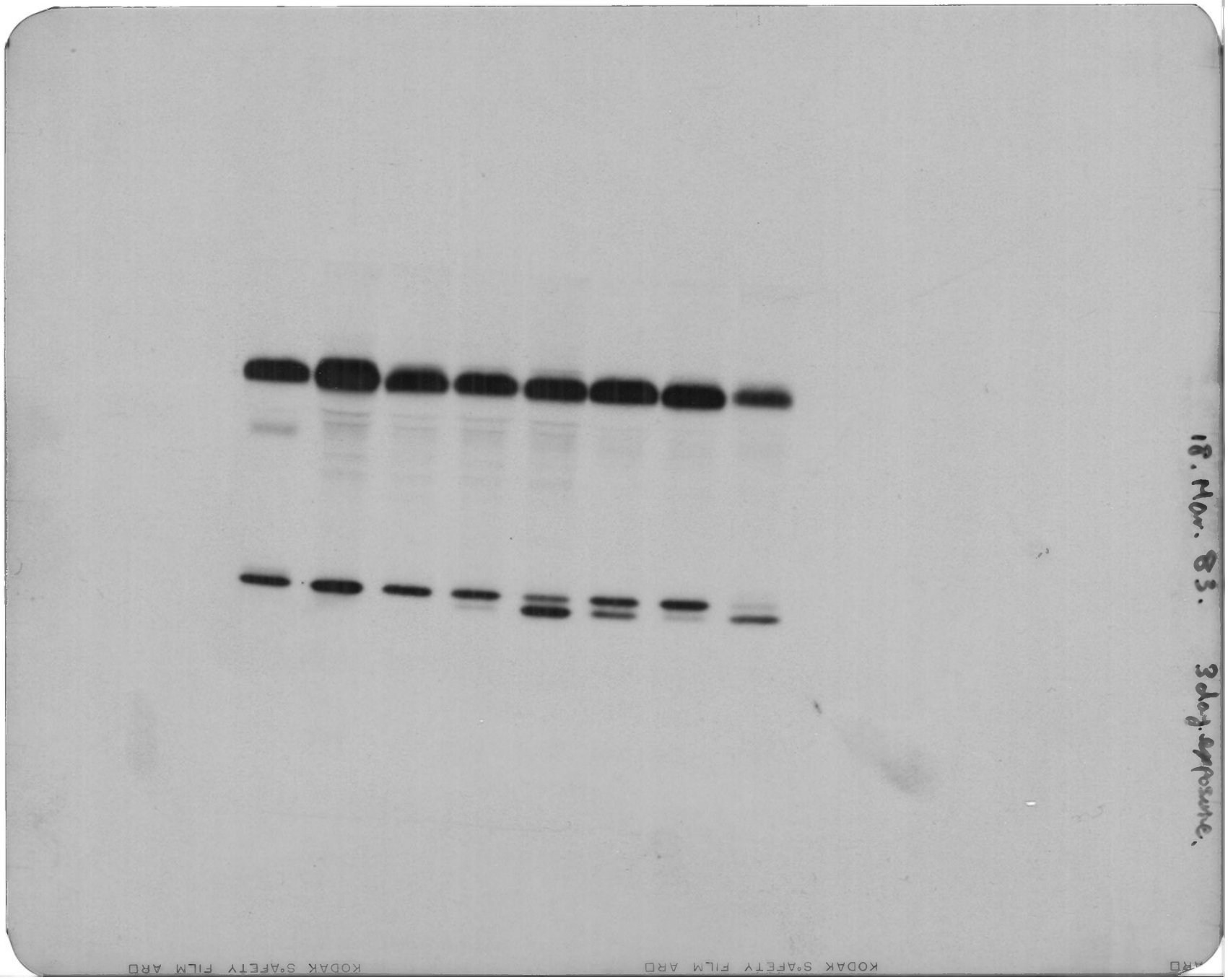
Staining of H-k Transformat 17 Mar 83

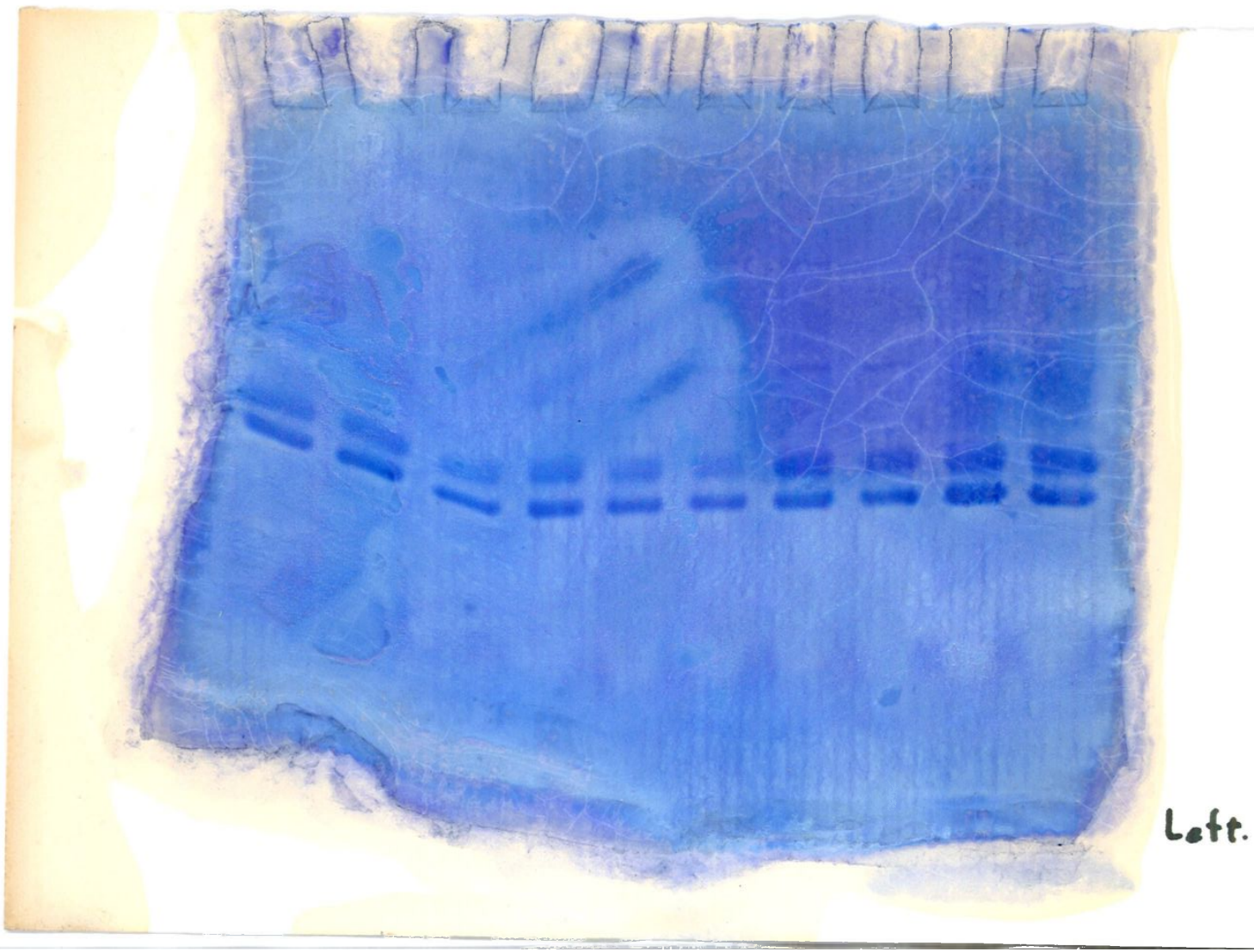
$\bar{o}$. $\alpha\mu^{FITC}$ 1:96.

Mouse pre-B (+)

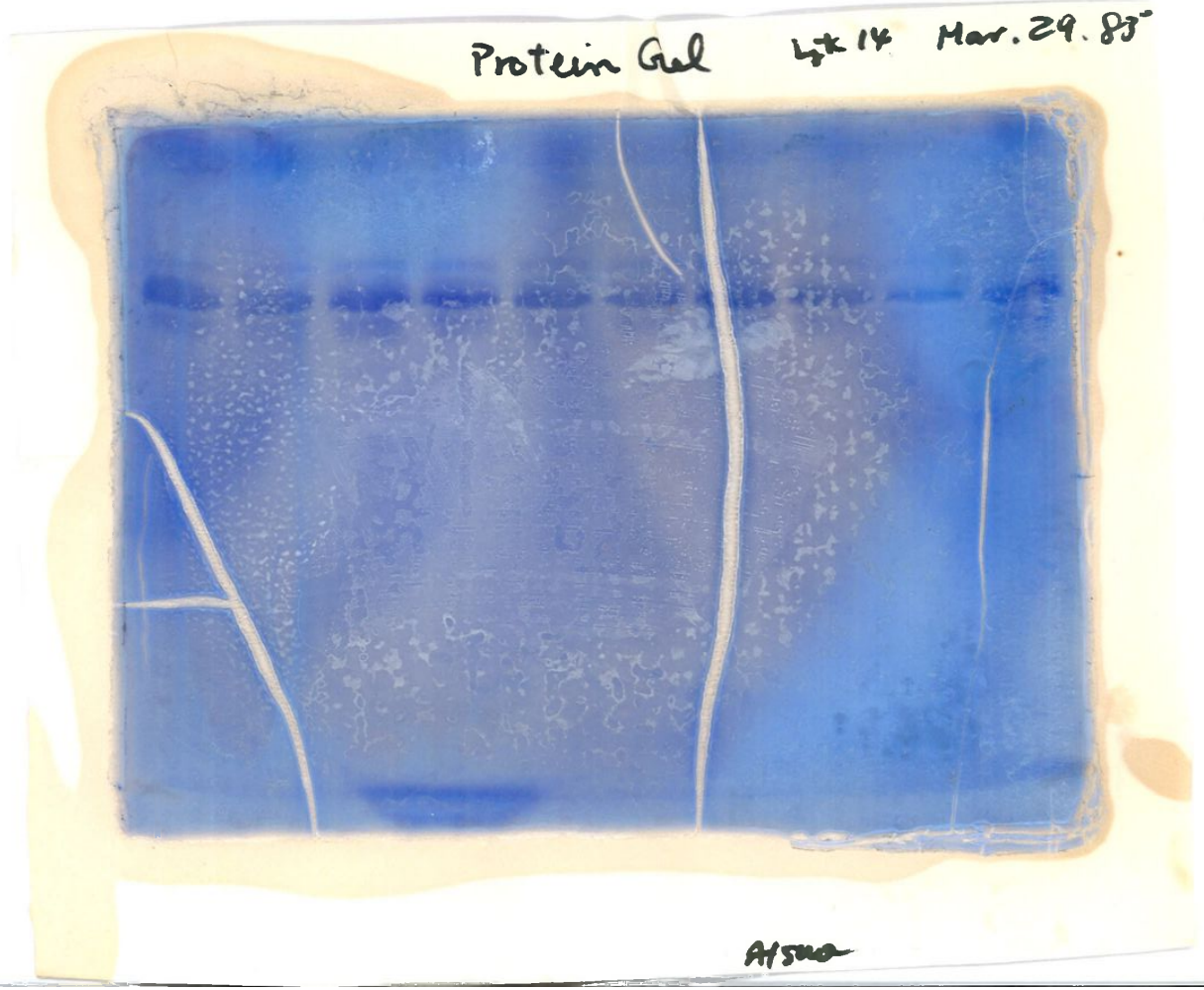
but all transformat x63, Sp2/0 } → negative.

Mar. 18. 83' light transformants


18. Nov. 83. 3 day exposure.
KODAK S°AFETY FILM ARD
KODAK S°AFETY FILM ARD

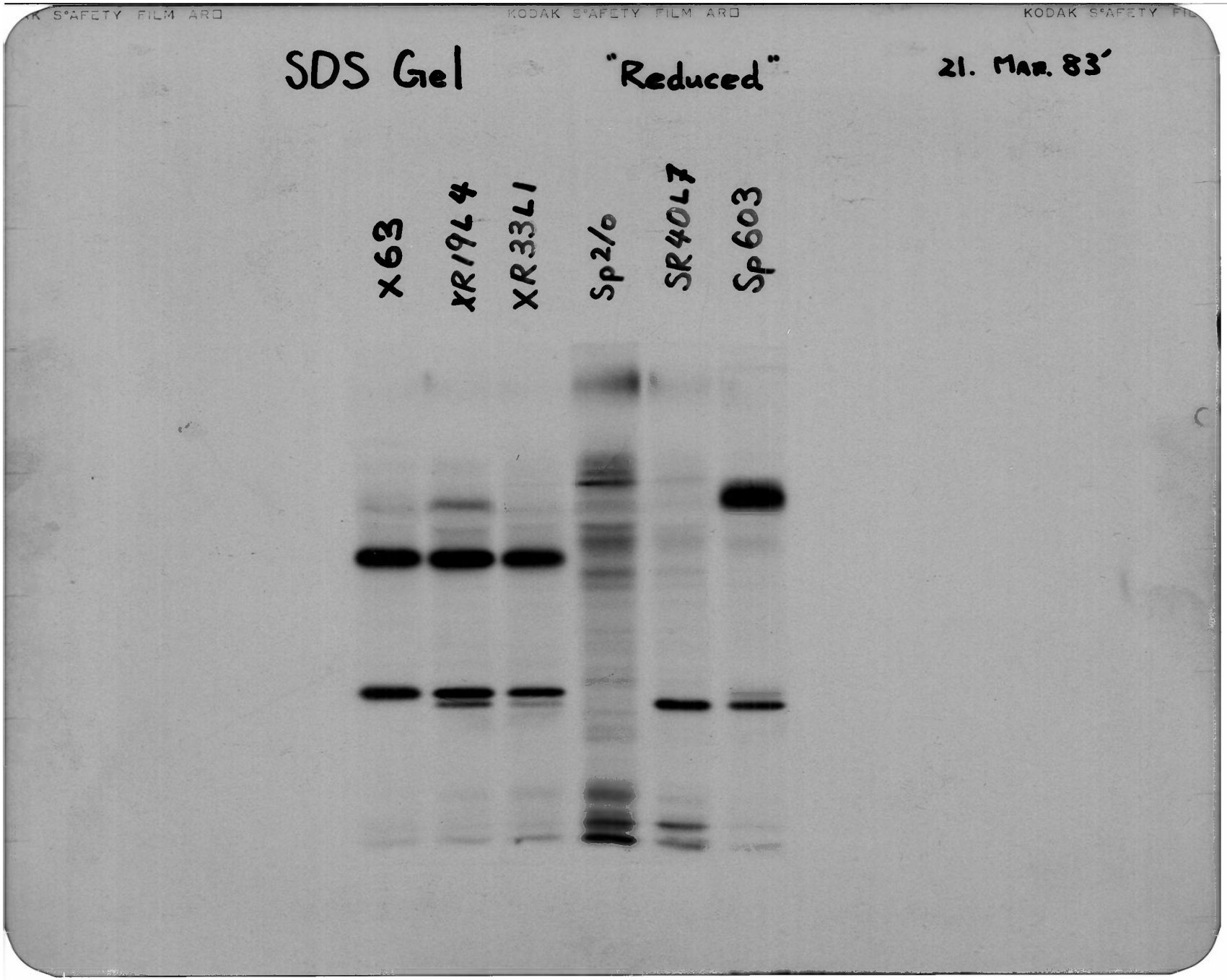

Left.

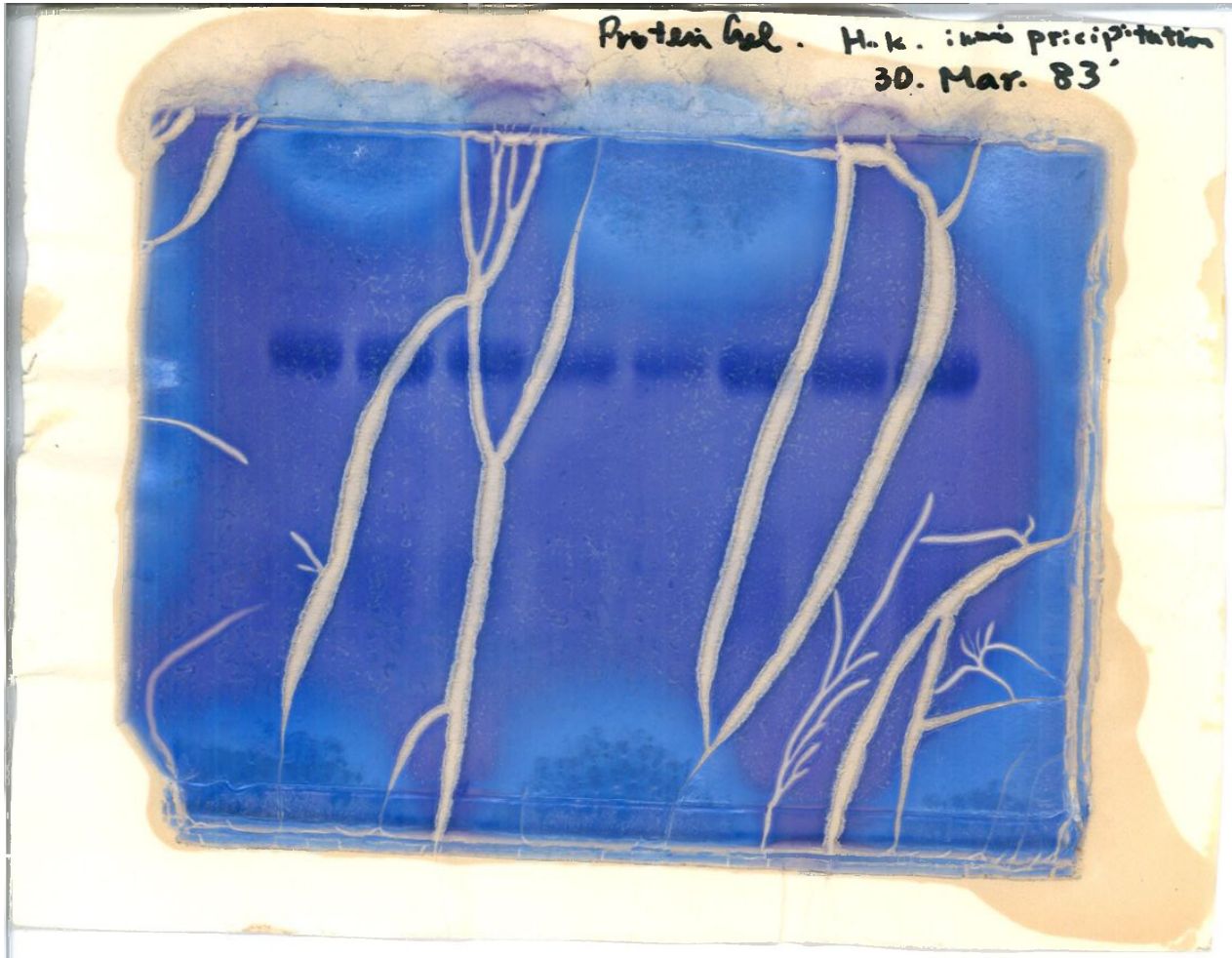
Unreduced Protein Gel exposure 7 days 21. Mar. 83'
x63 x63 Sp½ SP Sp
x63 L19 L33 60 603
KODAK SAFETY FILM ARO

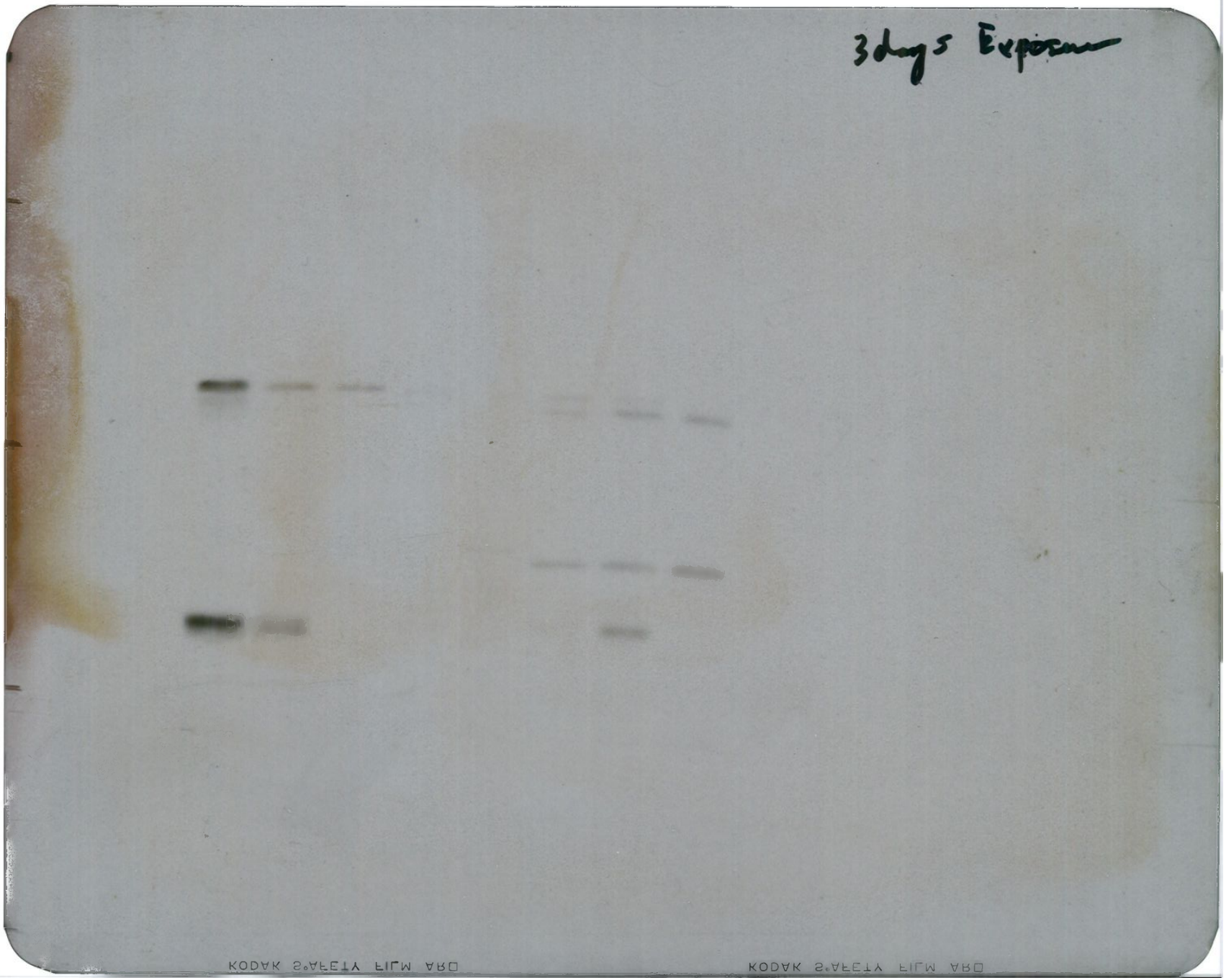

Protein Gel 4x14 Mar. 29. 85
AISMA

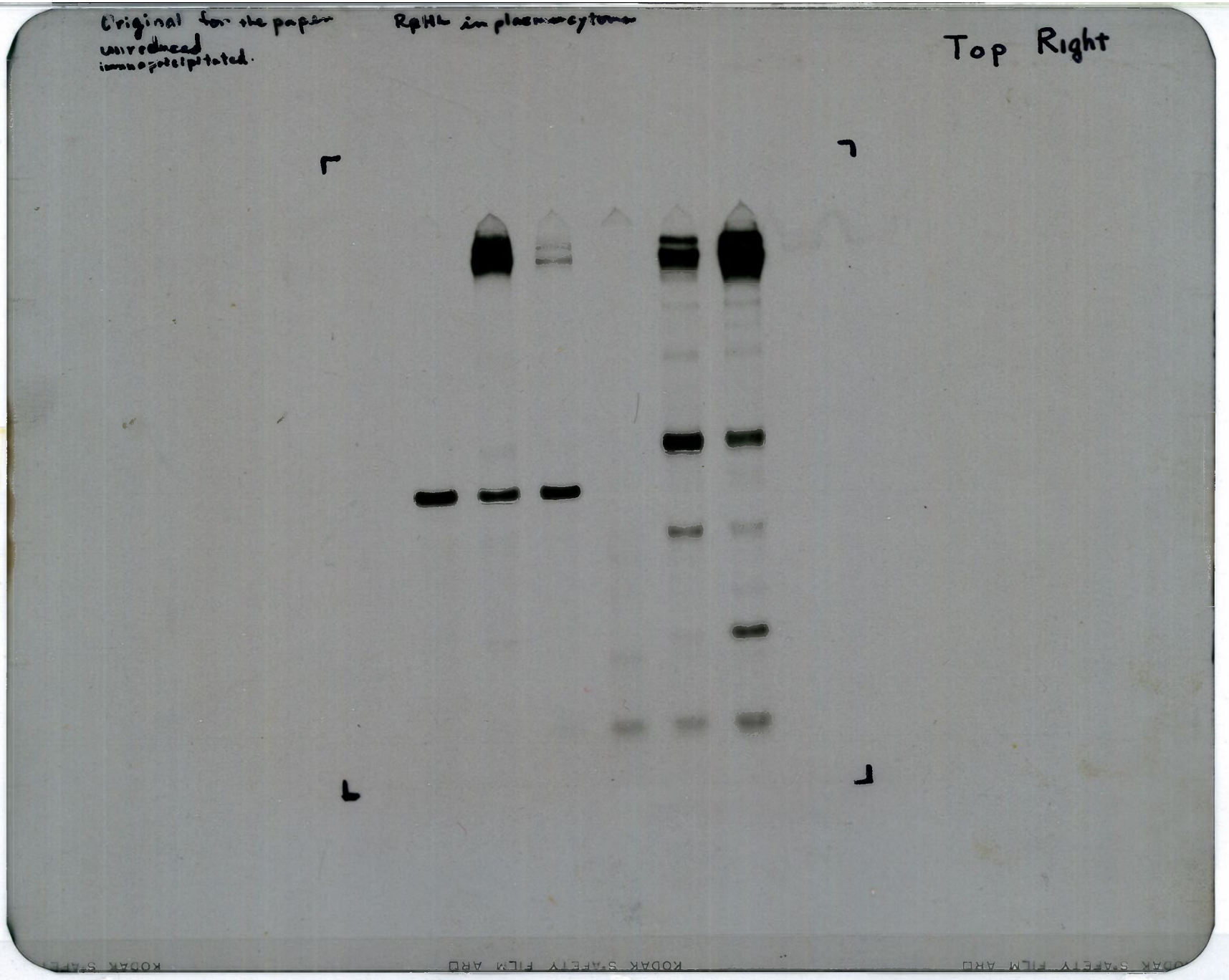
Top Right
KODAK SAFETY FILM ARO
KODAK SAFETY FILM ARO

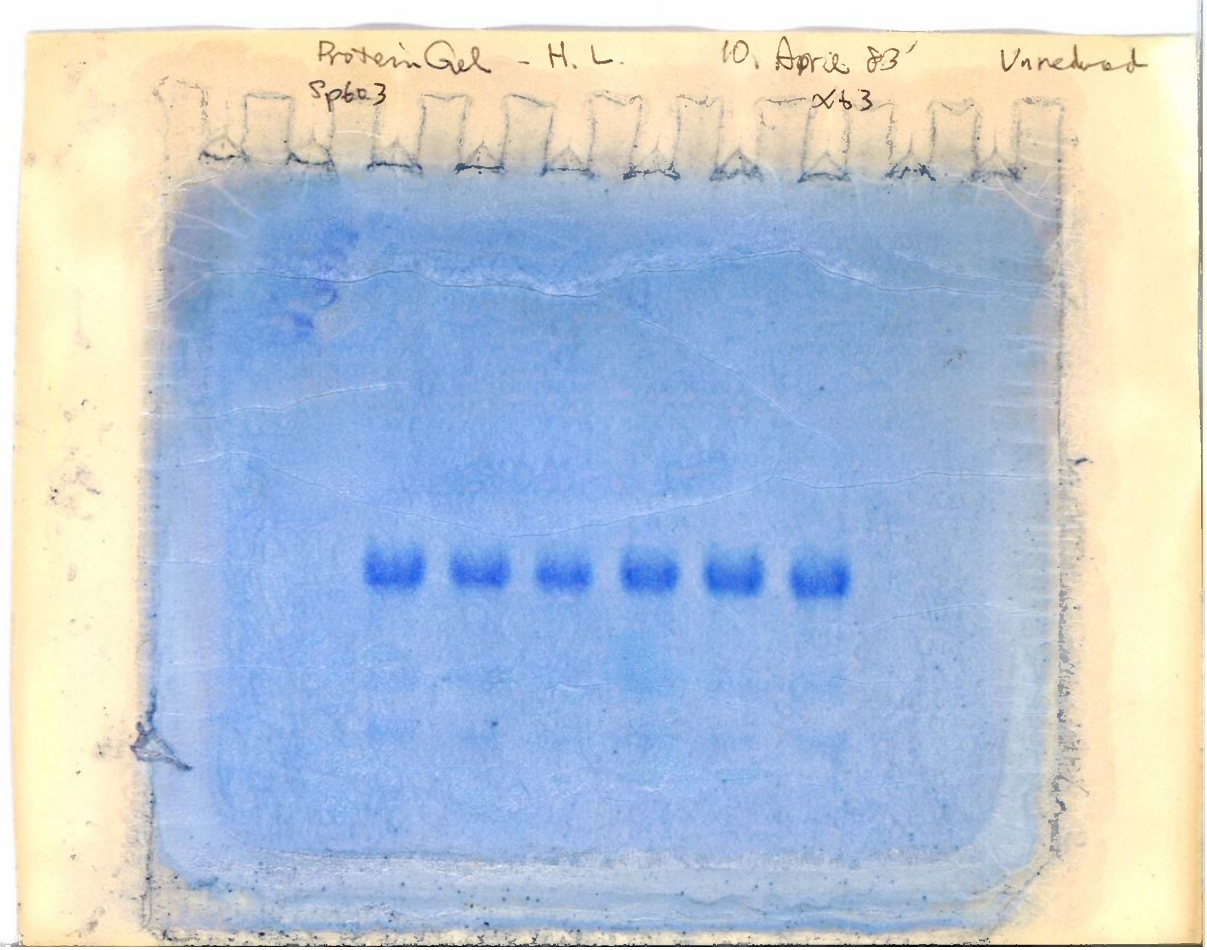
Exposure 2 days
KODAK SAFETY FILM 4489
KODAK SAFETY FILM 4489

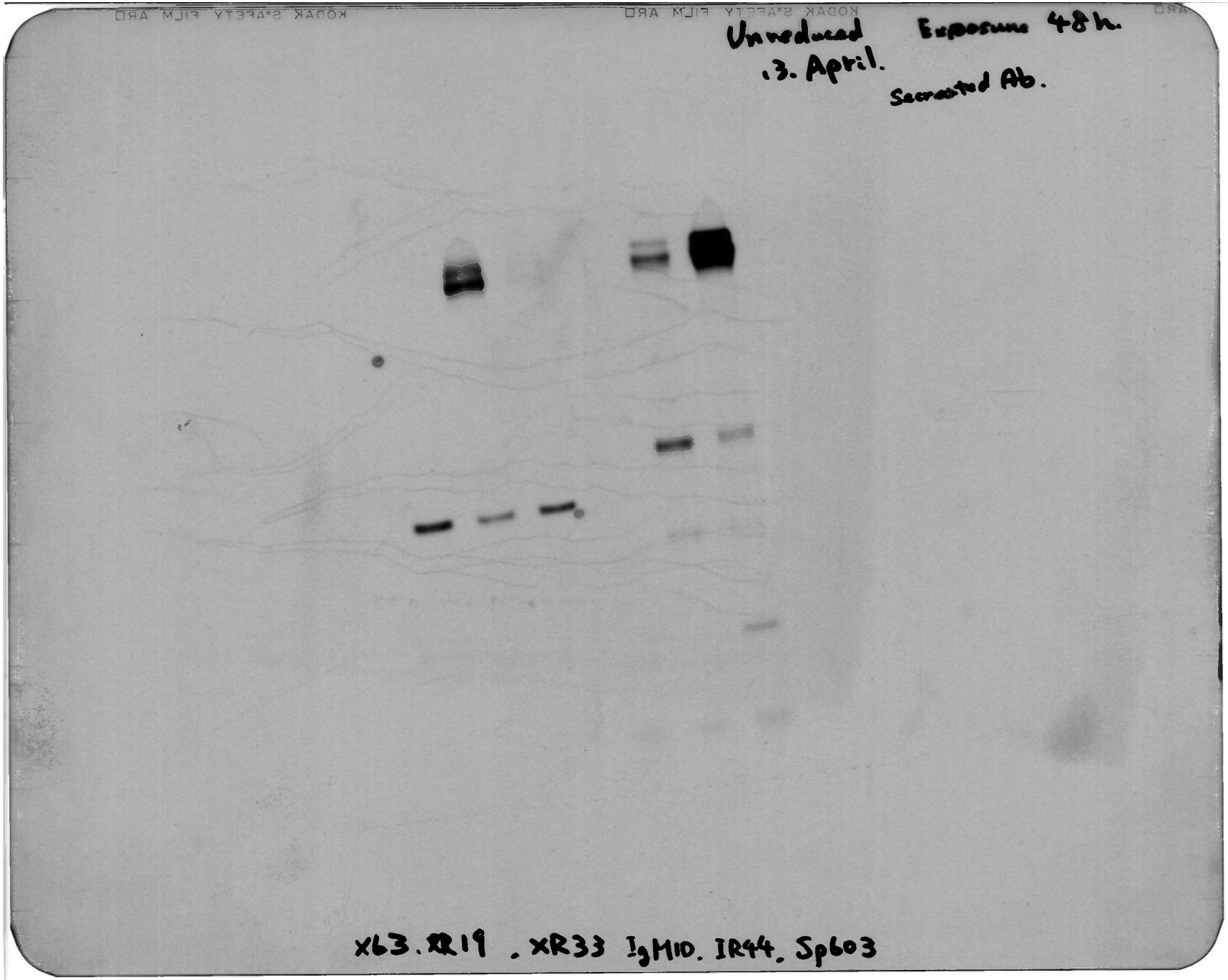

SDS Gel
"Reduced"
21. Mar. 83'
X63
XR1944
XR3341
Sp2/0
SR4017
Sp603

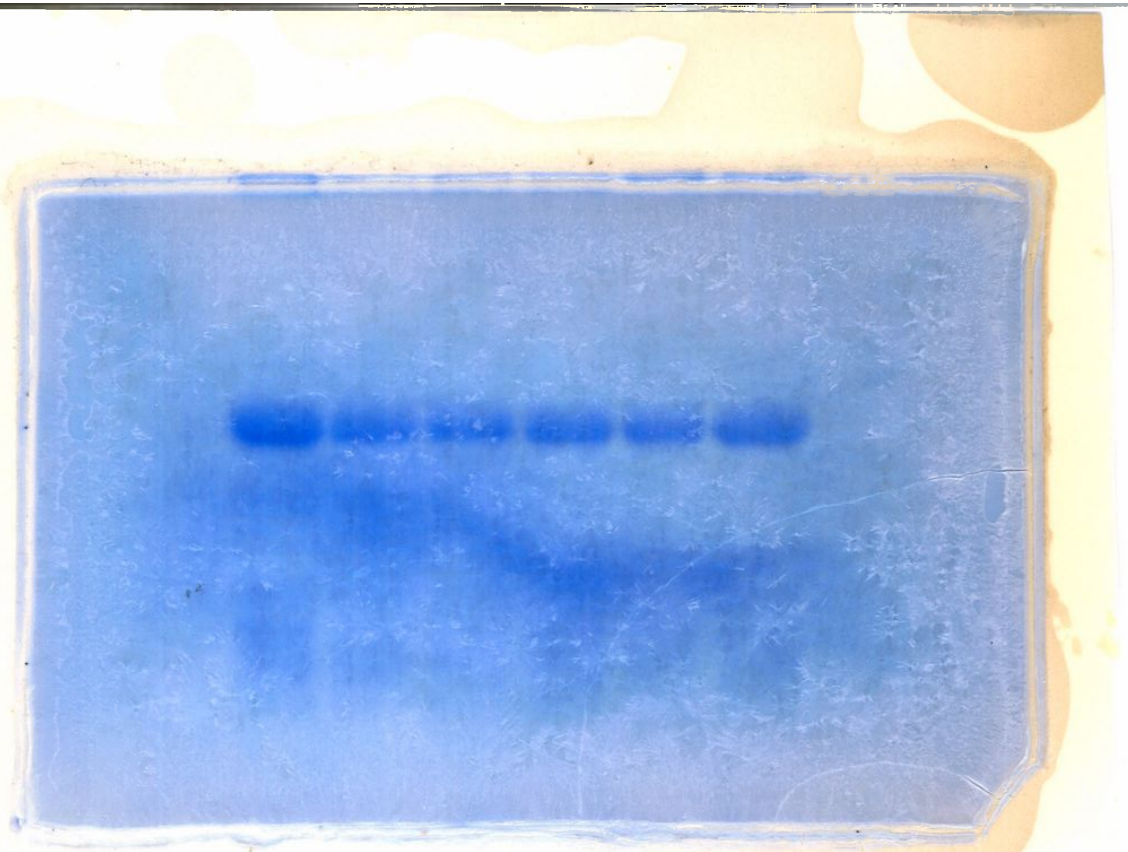

Protein Gel. H.k. immun precipitation
30. Mar. 83'

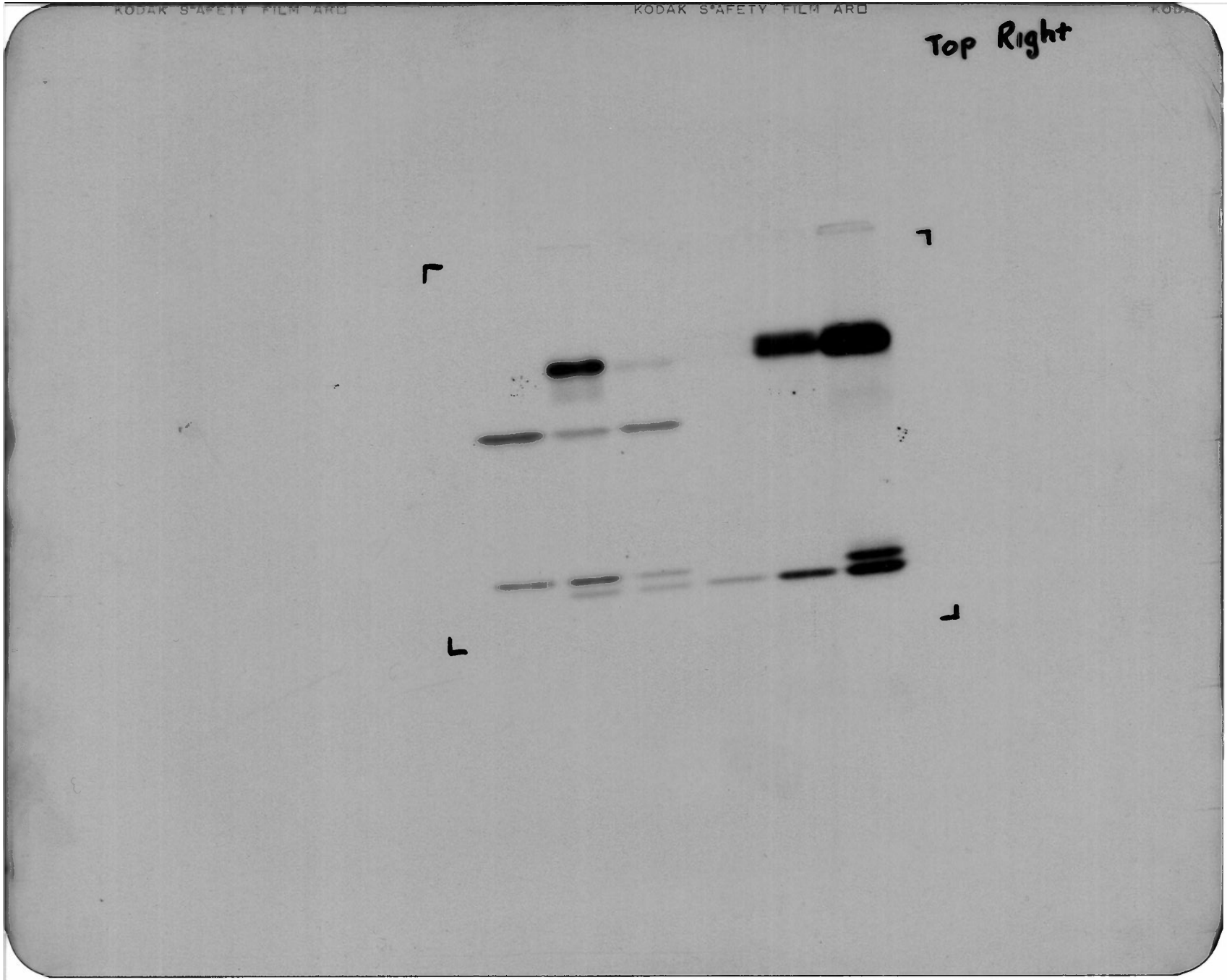

3 days Exposure
KODAK SAFETY FILM ARO
KODAK SAFETY FILM ARO

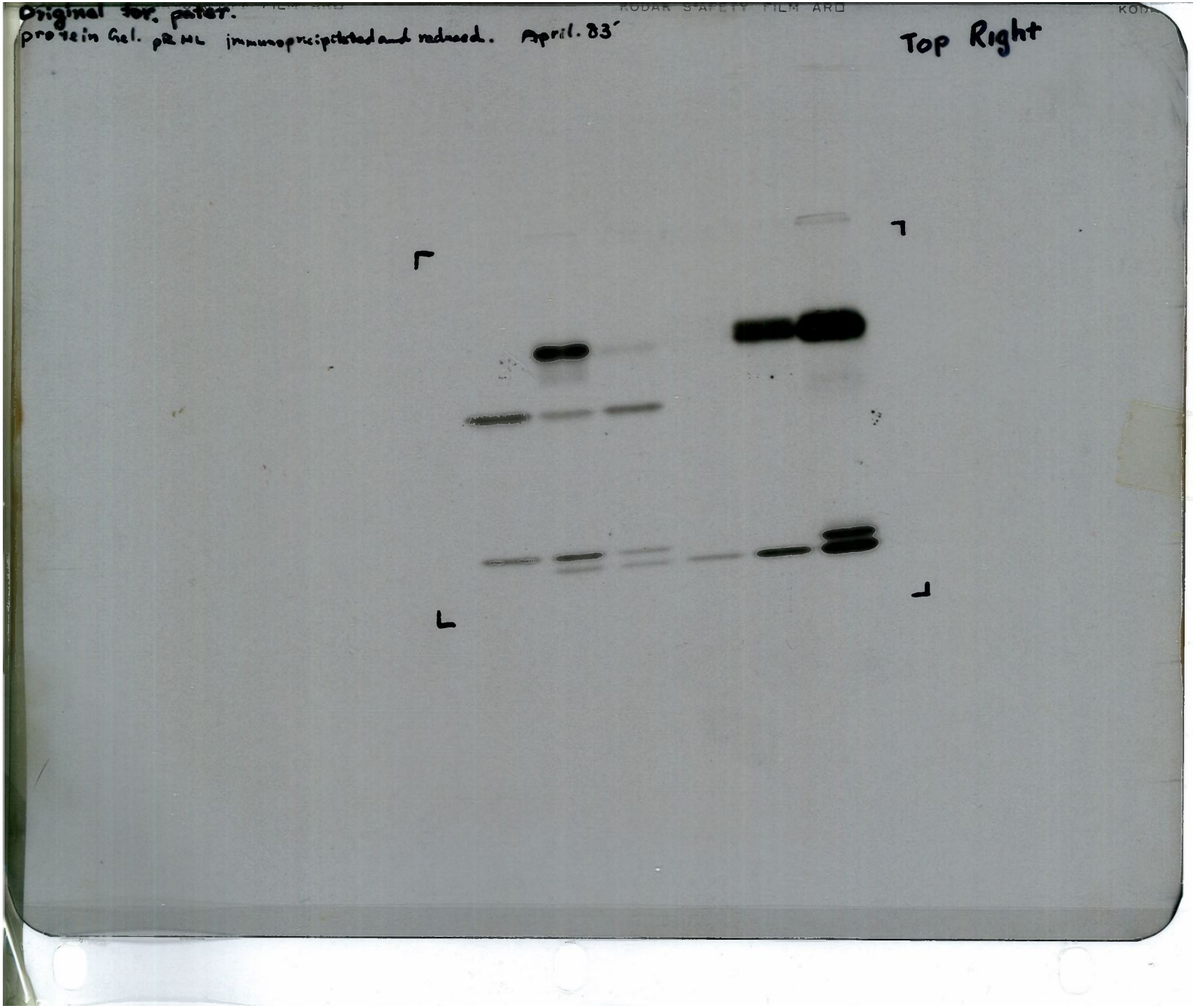

Original for the paper
unreduced
immunoprecipitated.
RpHL in plasmacytoma
Top Right


Protein Gel - H. L. 10. April 03' Unreduced
Sp603
x603


Unreduced
13. April.
Exposure 48h.
Secreated Ab.
x63. XR19 . XR33 IgM10. IR44. Sp603


Protein Gel H.L
10. Apr. 83
Sp603 IR44. IgM10 - XR33, XR19, X63
reduced and immunoprecipitated


KODAK SAFETY FILM ARO
KODAK SAFETY FILM ARO
Top Right


Original for. peter.
protein Gel. pRML immunoprecipitated and reduced. April. 83'
Top Right

10. Apr. 83

Protein Gel

Reduced and Unreduced.

Samples

Immunoprecipitated.

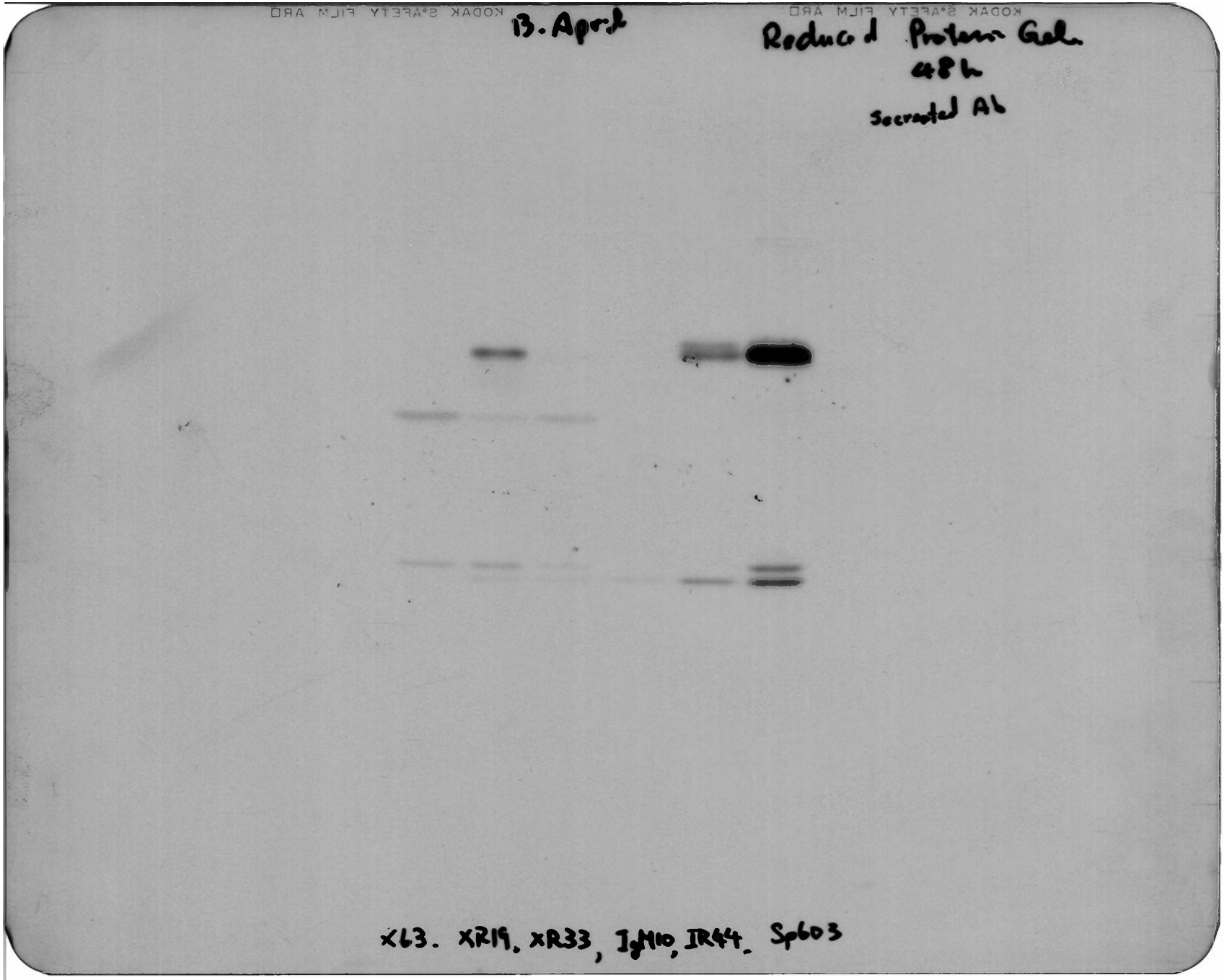
X63. XR19. XR73 IgM10. IR44. ~~Sp603~~.

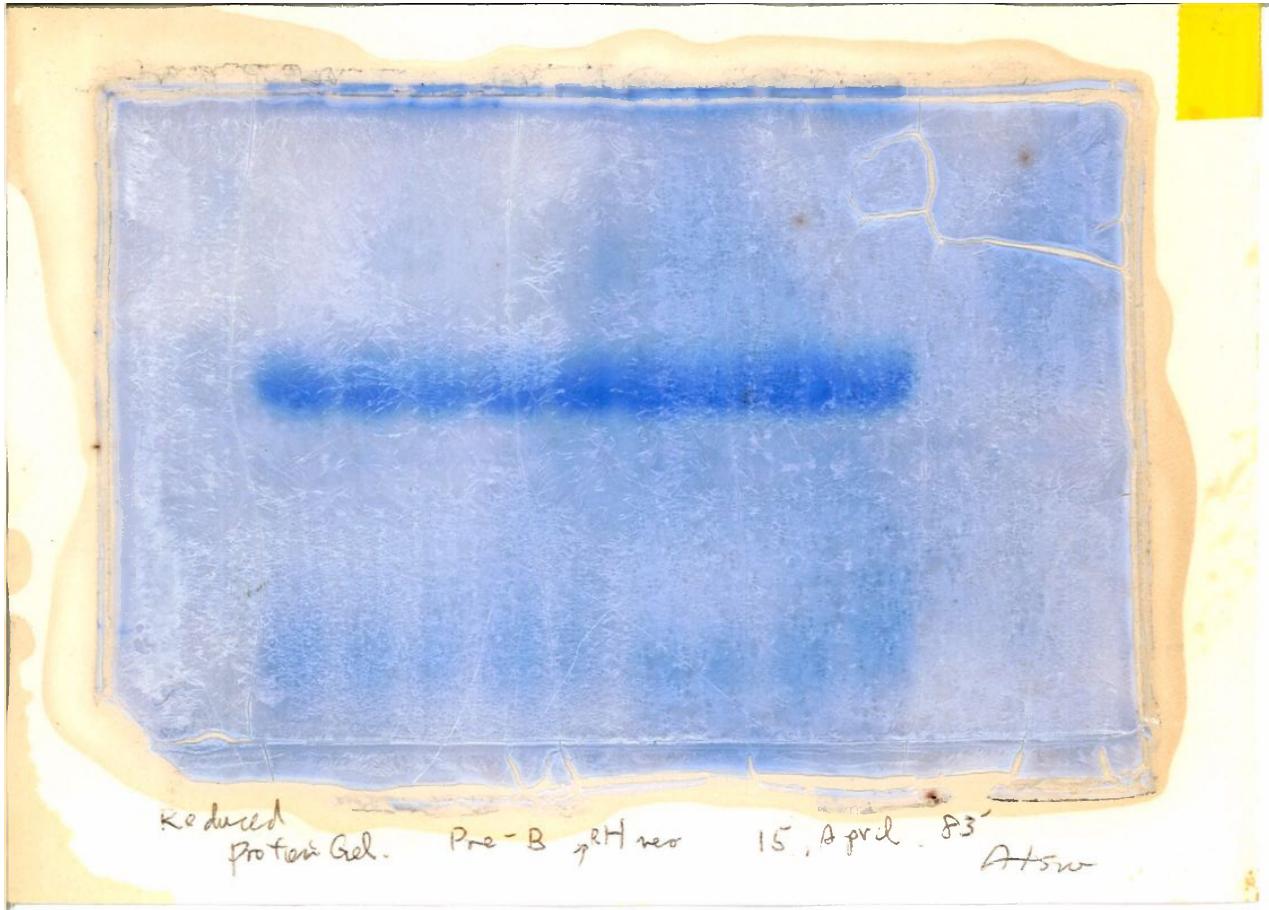
25λ. Rabit α μ (containg α Fab) — Protein A-Seph

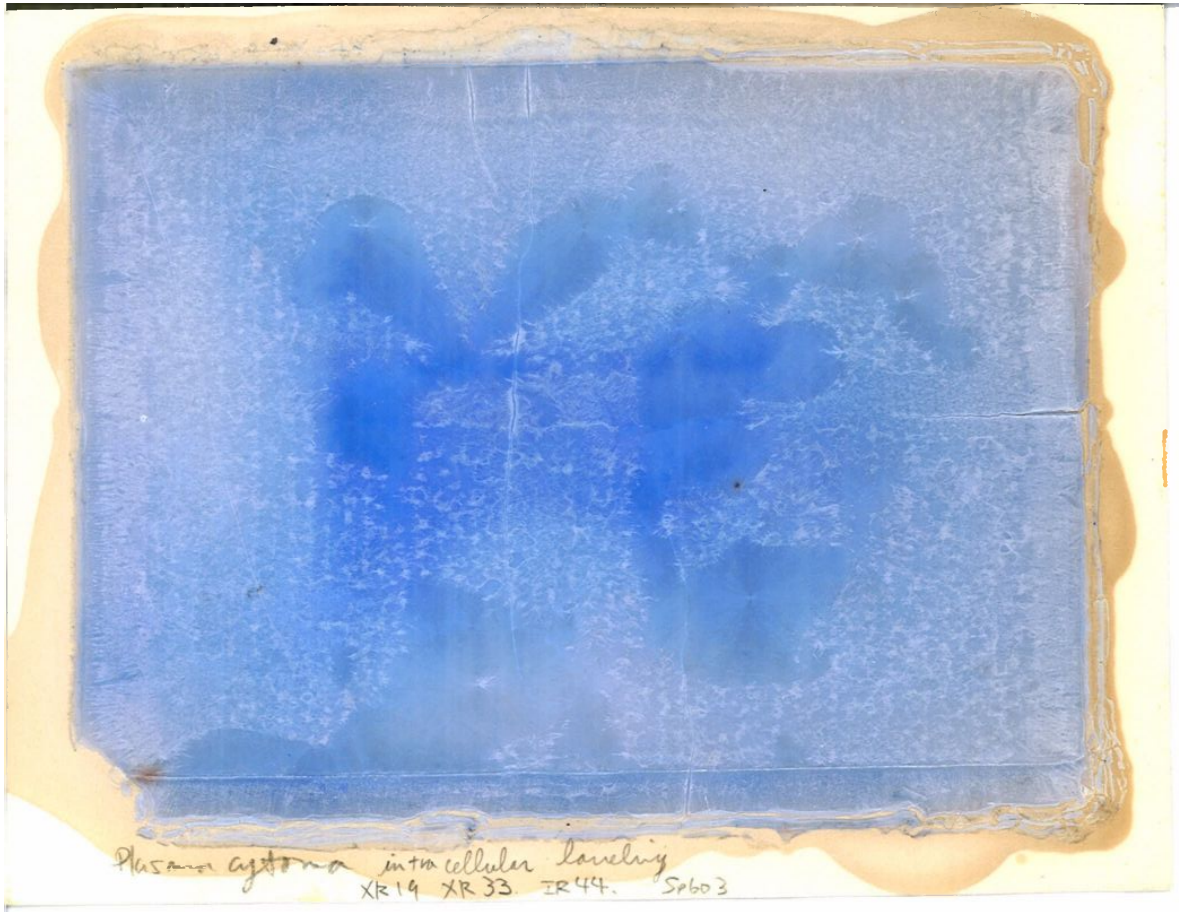
+ ~~10~~ 6 10λ Culture soup.

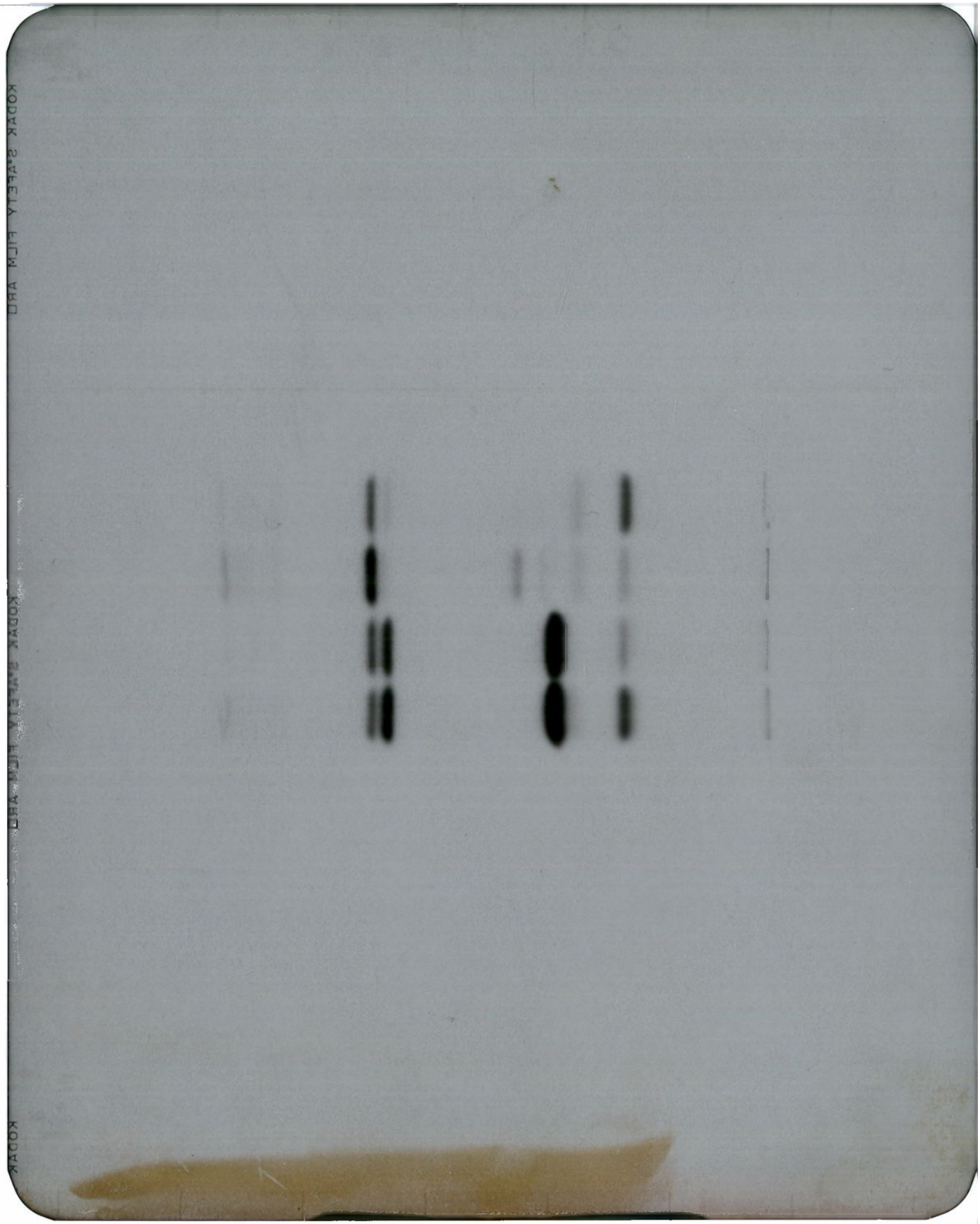
Reduced
3 min Boil
30 mA 1h

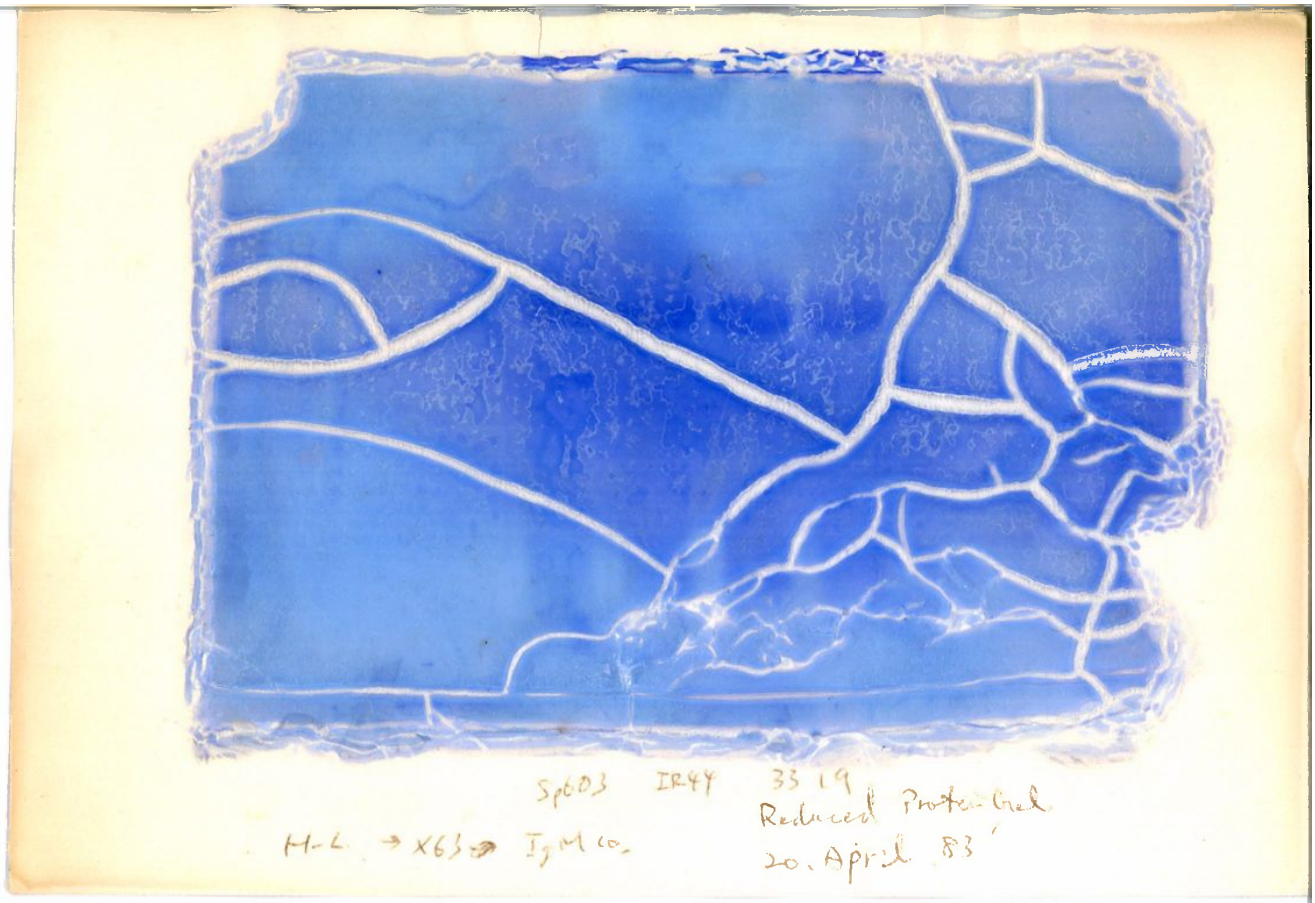
Unreduced
↓ 30 sec Boil.
25 mA. 6h

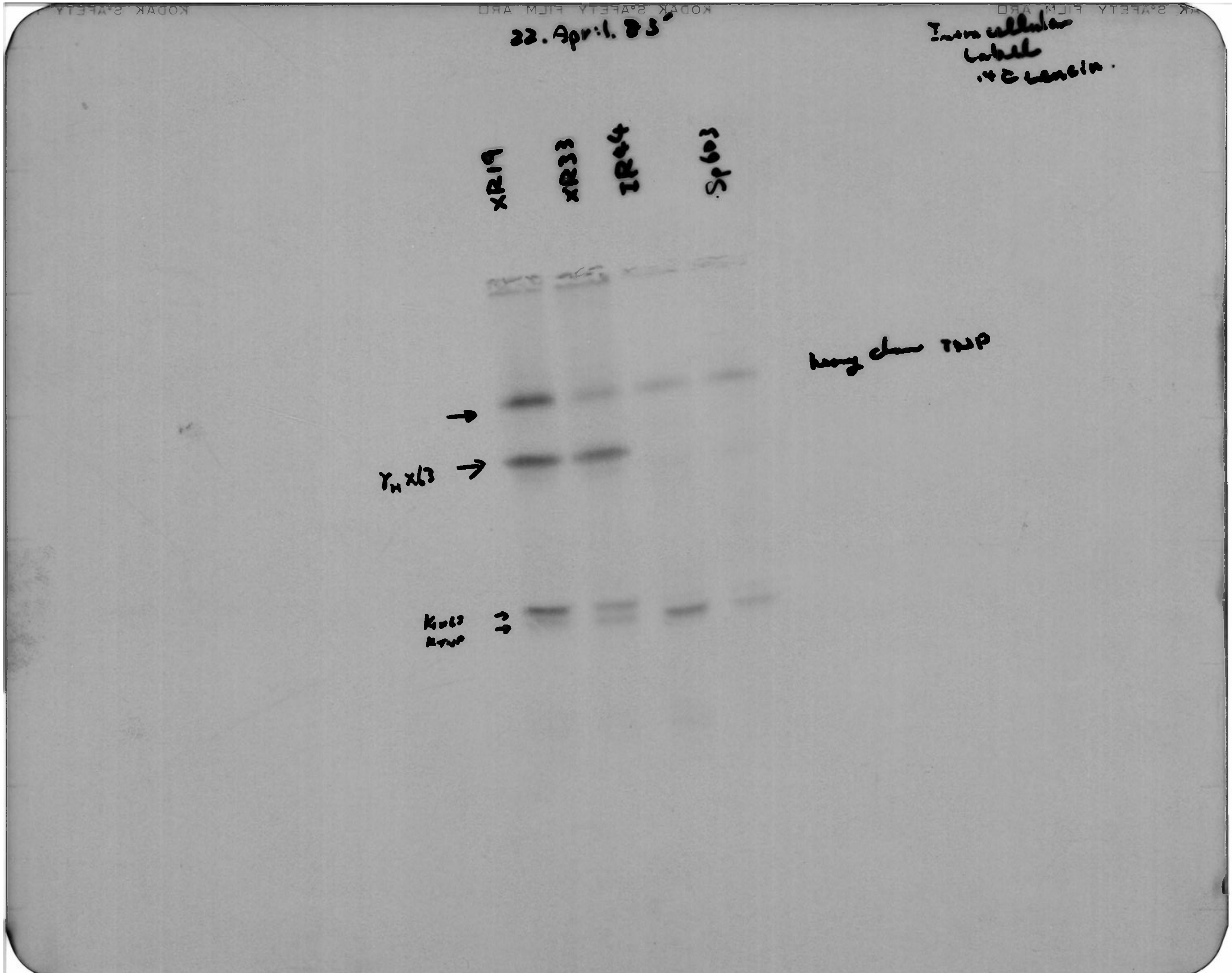

13. April
Reduced Protein Gels
48h
Secreted Ab
x63. xR19. xR33, IgM10, IR44. Sp603

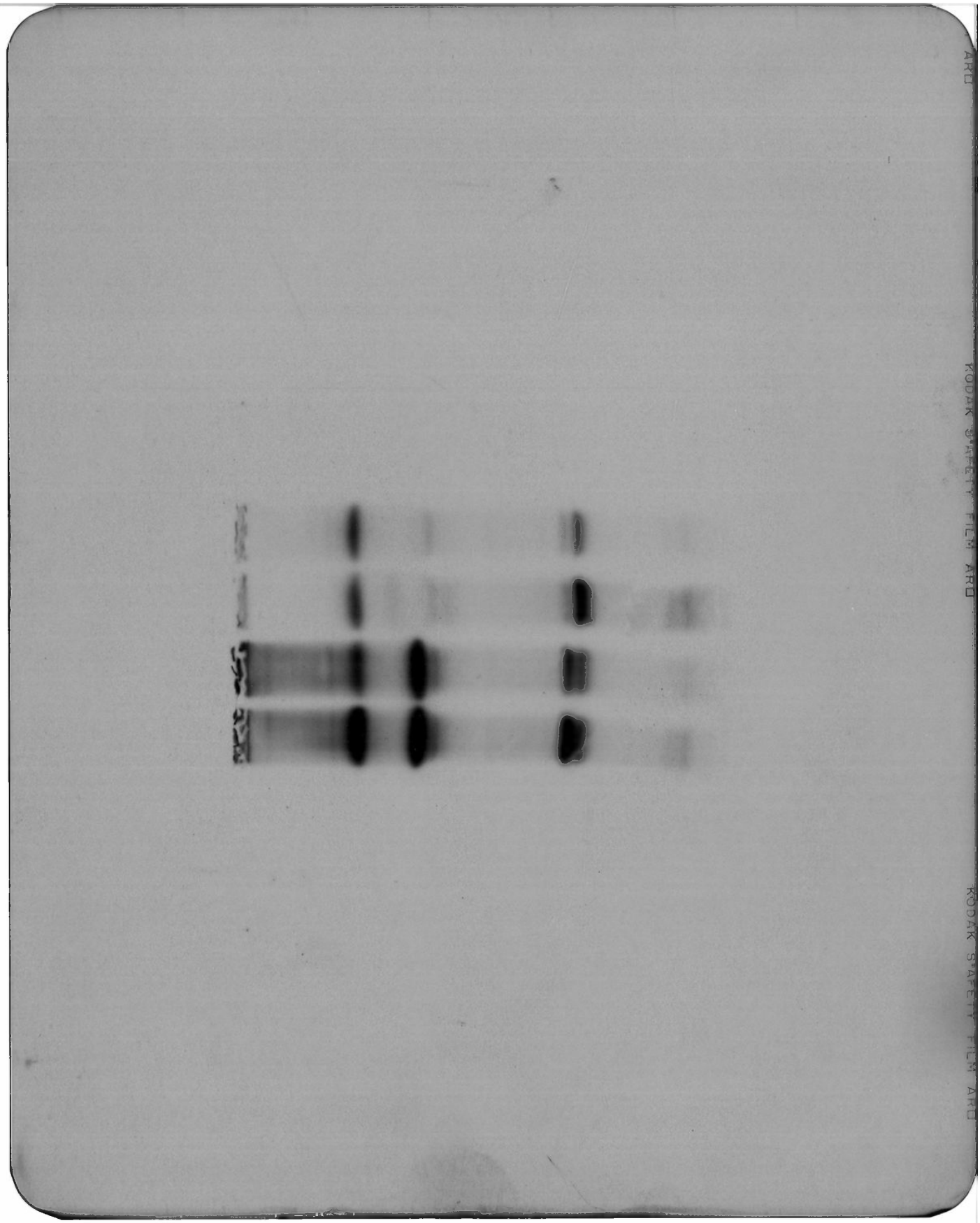

Reduced
Protein Gel. Pre-B pH neo 15. April 83
Atsw


Plasma cytoma intracellular labeling
XR14 XR33 IR44. Sp603


KODAK SAFETY FILM ARG


Sp6D3 IR44 33 19
Reduced Protein Gel
H-L → X63 → IgM Co.
20. April 83


22. April. 83
XR19
XR33
IR44
Sp6/3


KODAK SAFETY FILM ARO
KODAK SAFETY FILM ARO

Protein Gel 18. April 83'

15mA → 30mA 4 h

Samples ~~XR9~~ XR19 XR33 JEKR 0603.

Summary of gene transfer of pR-HL neo to mammalian cells

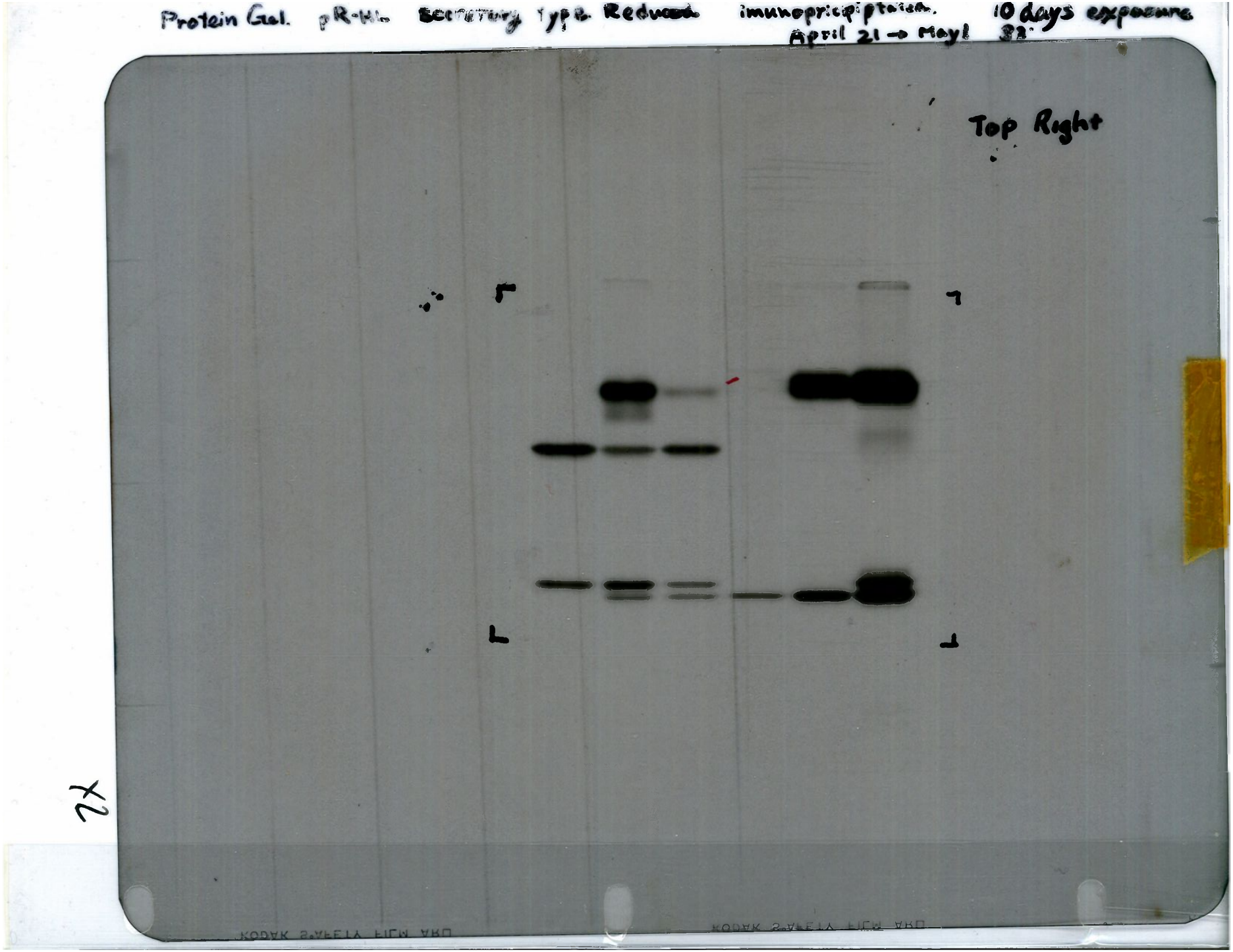
Reciepient cells

		try 3 times	total
X63-Ag8		3	⊕15/12/432 wells
Sp2/0		3	2/9/432
XNP	8-653	2	2/3/288

fusion condition 8×10⁶ cells : 10⁹

24 wells 10⁵/well 10⁴ ~ 5×10³/well 9 wells

L cell	$10^{-2} \sim 10^{-3}$	10
CV1 cell	$10^{-2} \sim 10^{-3}$	10
BW5147	weak to G418 0.5 mg so for 5 clones.	

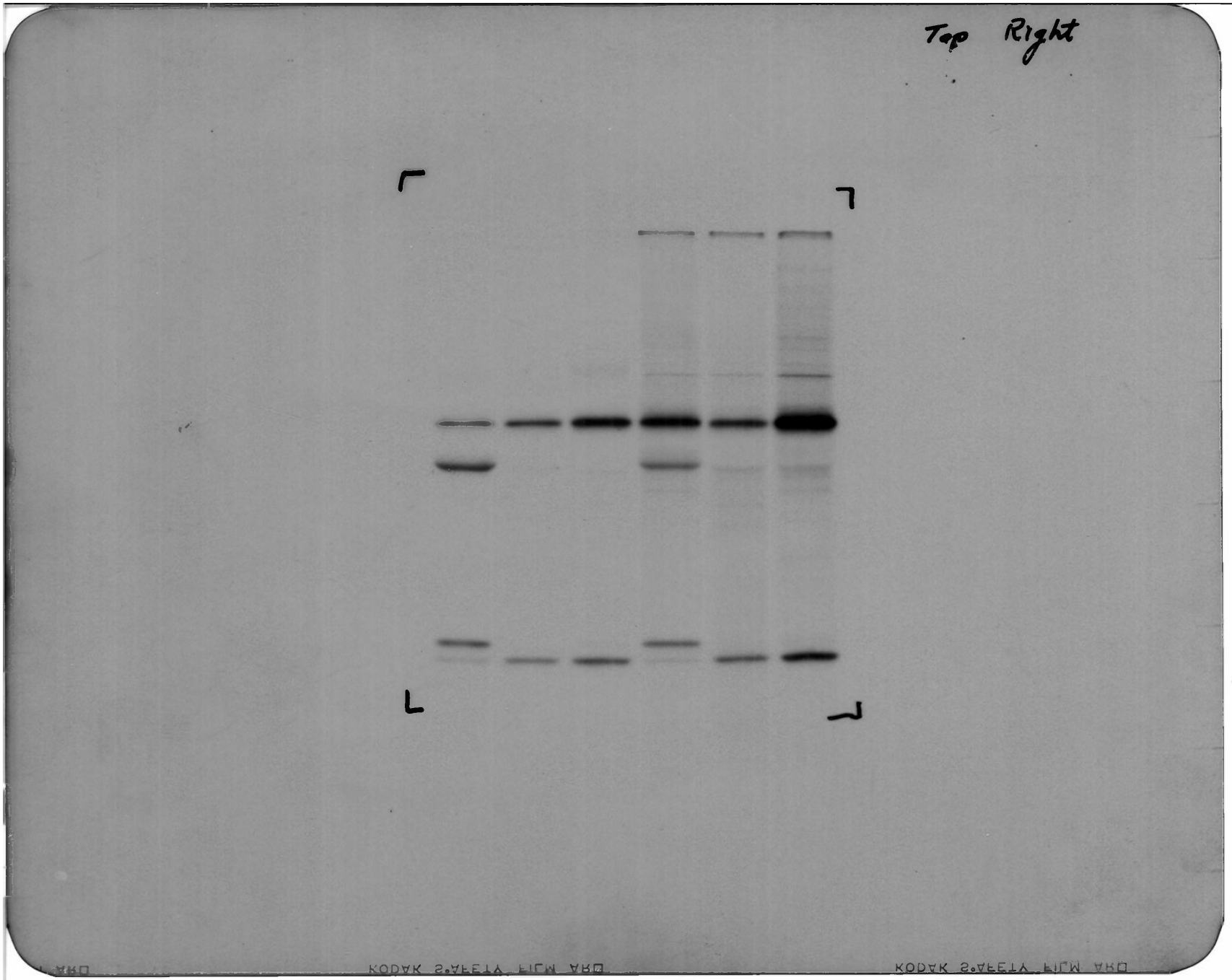

Protein Gel. pR-HL Secretory type Reduced immunoprecipitation.
April 21 → May!
10 days exposure
Top Right
2X

12 May, 83'

Measurement of a TNP antibody in culture soup of H or HL chain gene transfered clone

			% of
Cell	Sp603	10240	100
	XR19	1280	12.5 %
	XR 33	320	0.375 %
	IR 44	2560	25 %


plasmacytoma Tunicamycin, treated
29. May. 83
intracellular and


Top Right

Dexter culture 1

Aug. 30 82

Experimental animal BALB/c J 5heads

Born marrow cells Femur and Tibia.

241 × 2 × 2 × 10^6/ml

total. 9.6×10^7

Protoplast. 1×10^9 803 No 4 (reverse)
pSV2neo TK1

PEG. { A sol 15 sec
B sol 15 sec

no spindown.

no feeder layer

distribute to 24 wells. 4×10^6/well

Dexter culture No 2 Sep. 1 82

Dexter Culture 3 3 Sep. 82

Experimental Animal BALB/c 4 heads

Bone marrow cells Femurs and Tibias

8.1×10^6 /ml 13 ml

1.05×10^8 total.

Gey's solution 45 sec.

↓

wash × 2.

↓

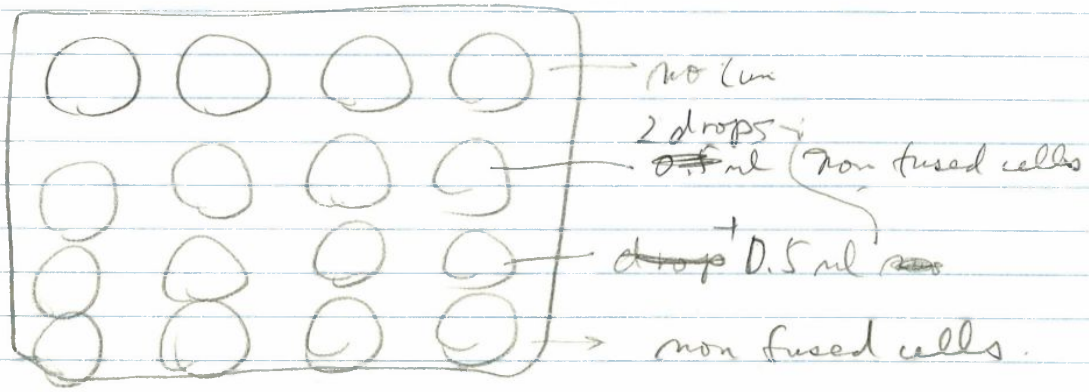
Fusion. (A) solution. 20 sec

↓

spindown

↓

distribute 24 wells



Gene transfer ~~to~~ born marrow cells.

Experimental animal . BALb/c

born marrow cells . 2.5×10^7/head

strategy : Born marrow cells (4 heads) → 2 plate 48 wells

↓ 1W

feeder layer cells 0.2 mg/ml Neo

(2 heads) ( ) Gene transfered born marrow cells → ↓ 1W

neo resistant feeder layer cells. 0.2 mg/ml Neo

(2 heads) ( ) Gene transfered born marrow cells → ↓ 1W ~ 2W

colonies . 0.3~1.0 mg/ml

8 heads + 4 heads 12 heads

4 heads of mice (BALb/c)

↓) 18-24h. irradiation. 600 R

(2 heads) Gene transfer ~~2.5 x 10^7 cells~~ /head (1×10^7) x 4

5 days (1×10^7 /heads 4 heads

hybridize {LN — ~~BW~~ ; Spleen < BW, X63 } neo myc Selection

Oct. 25.

Preparation of feeder layer Dexter culture

4 heads of CBA mouse

8.4×10^7 total

dilute 40 ml ∴ $\div 2 \times 10^6$/ml

2×10^5/well → 96 well plate

4 plates

New protocols for Protoplast fusion

Improved point.. pH of. {PEG 0.84 g. DMSO 0.2 g
MEM 1.0 ml.

add NaOH in water.

to the cell mixture plet,

add 0.1 ml of FCS and mix well

after the exporsure to PEG

dilute to 10 ml of FCS

↓ 40 ml of 20% FCS

from the first time cells were maintained in 0.1 mg/ml neo

Oct. 30 82

D-F-1 (Dexter Culture No 1)

Dexter Cultured Cells Sep 27 → ~~to~~ Boost ×2
Small flask : from Ken

Collagenase treatment 20 min at 37°C.

Detach cells c pipeting 8×10^7 total

4×10^7 4×10^7 ↓ Fusion

PEG 1.0 g + 0.3 ml of DMSO.
+ 1.7 ml of D-MEM adjust pH c NaOH 0.1M)
33 %

(1.5 ml of PEG) Mix for 15 sec → dilute to FCS solution (10 ml)

After the fusion cells were mixed c non PEG treated cells and distribute 0.125 mg/ml G418 medium 20 ml

Oct 25 ~~feeder~~ layered directly on 96 well plate

Control C14.
2000 ~~cells~~ / well 96 ~~well~~

2. Nov. 82

Dexter Culture No 2

{ 2.9×10^6 /ml 13ml — Dex
2.5×10^7 /total 11ml — B

D-F-2

Fresh born marrow cells CBA/J

Dexter Cultured cells Sep. 27 —

Fresh born marrow cells 2.5×10^2

Dex — 4×10^2.

PEG 1.0g + 0.3ml of DMSO + 1.7ml of D-MEM

{ Dexter Culture . 20 sec.
Fresh born marrow cells 15 sec.

Dex → 1/3 → Fusion and no feeder layer on other dex culture
→ 1/3 → { fusion and mix with other 1/3 cells
feeder layer . feeder layer

Born marrow cells → fusion and → no feeder layer cells
→ feeder layer cells

0.25 mg/ml G418

9. Nov. 82

Dexter No. 4.

Strategy. PEG 40%.

1 ml + 1.5 ml (~~0.25~~ 0.25 ml DMSO + 1.25 D-MEM)

~~0.5 ml~~ (1 ml)

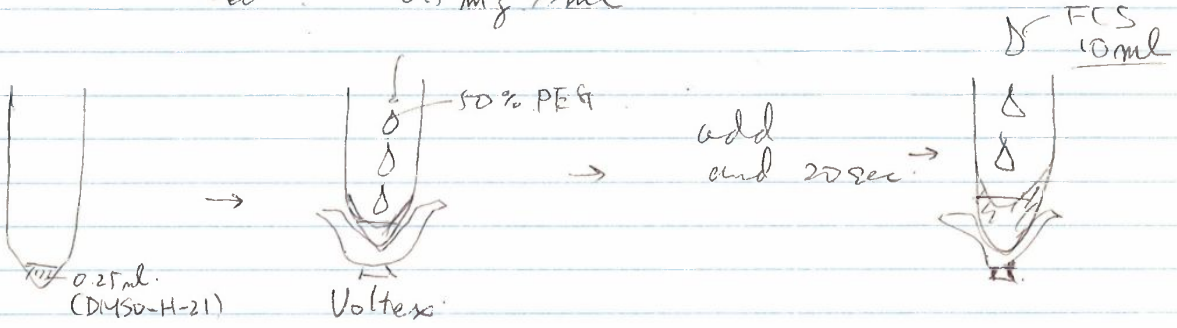
1 ml 0.75 1.25

~~66% PEG~~

50% PEG. 1 ml

0.25 ml (DMSO+D-MEM) → cell pellet

1) eo. 0.5 mg/ml.



Result — Bad.

Viability is terrible

1 ml

1 + 2.1 (0.4 × 2.5)

0.4 + 0.1 = 0.5

Dexter 5

1 Bottle → 33% PEG 10% DMSO
20 sec.

dilute to 10 ml FCS
↓ no incubation
wash × 2
↓
distribute 1 96well plate

Nov. 15.

Check of PEG Sensitivity

no pipet { 1. ~~10% DMSO 33% PEG 20 sec~~
2. 10% DMSO 40% PEG 20 sec

1' dilute to FCS

2' dilute to D-MEM

Procedure.

Cell harvest → spindown → add enough amount of D-MEM → spindown twice

PEG KO % { 0.84 + 0.2 DMSO + 1 ml H-21
0.84 + 1.2 ml H-21
→ 37°C warmed.

add gently · shake with tapping · for 20sec

add 5ml of FCS (warmed) + then 5ml D-MEM
D-MEM (") + 5ml D-MEM.

↳ Spindown and resuspend FCS
↓
distribute · 96 wells

48 h.

DMSO ⊕ group has many dead cells.

but · DMSO ⊖ → FCS suspended group was at good viability.

Nov. 17

D-F-6

cells — Dexter culture from ten.
CBA/J

2×10^7 cells → 125 ml { Bacteria standard 30 G

0.05 ml and mix.

0.8 g PEG + 1.0 ml D-MEM 37°C

↓

0.45 ml

↓

add ~~cells~~ and 20 sec. mix

then add 10 ml warmed FCS
gently spindown

↓

0.2 mg Neo FCS

Protoplast
30 min
↓ Spindown
wash × 2 ← { 1 ml / 5 ml
↓
plet

cells
spindown
↓
wash × 2
↓
spindown onto the plet.

28. Nov. 82

D-F-8

half → 40% PEG 10 sec
↓ 33% PEG 10 sec

1 g + 1.5 ml 40%
1 g + 1.5 ml 33%
~~0.5 ml~~
0.6 ml 50 %

0.84 + 1 ml → 0.85 → 0.8 ml

1.0 + 1.8 → ~~0.6~~ ~~0.55~~ 0.65 → 0.5
~~5.1~~ 0.5
1.0 + 1.8 → 5.5 → 0.5

		DMSO	D-MEM	cell pellet
33%	10 sec	1.0 +	1.8 → 0.55	0.05 ml
40%	10 sec.	0.84 +	1.0 → 0.85	0.05

G418 . 0.4 mg

50% PEG . 1 g + 0.8 ml
1.8

15 sec.

D-F.-19. 5. Jan. 82.

40 % PEG. 15 sec.

5 ml p.-L.-Arg.

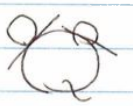
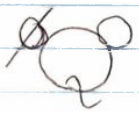
diluted à. dialyzed FCS

non- " FCS.

Vector pR-TK1

D-F-21

in Vivo experment.

1		control	H-21
2		pR-TK1 ↷ FBM	5×10^5/head
2		H-pR ↷ FBM	"
2		pR-TK1 ↷ Dex	6×10^5/head
2		H-pR ↷ Dex	"

↓

Fusion 26 Jan

Many Colonies in FBM

~~Reasonable No in 20% 5~~ Dex

~~20 30 Sec~~ 30 Sec in 40% PEG & 10% DMSO.

5 mg poly-L-Lysin

heavy chain mixed BM + Dex heavy

?

Light

28 Jan split to 2 wells

↓

1 ml 10% FCS feeded

O-F-21. 19. Jan. 83

Dexter culture. 2 small flasks.

40%. 20 sec. 5 µg Arg.

D-F-22 23 Jan. 83

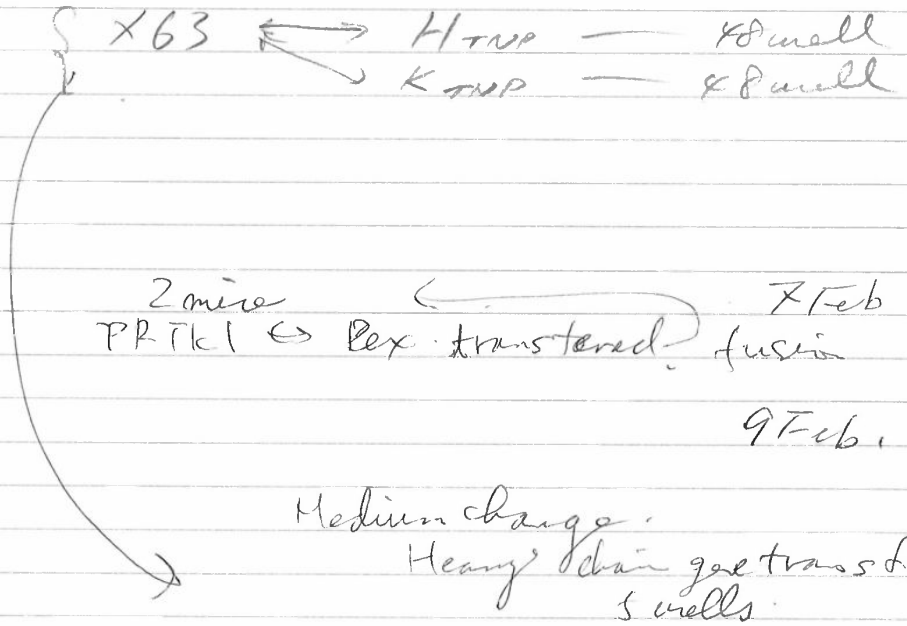
40% PEG - 5µg poly-L-Lysin
DMSO 1 drop
15 sec.
FCS dilution

D-F-24.

40% PEG. 25% Glycerol. Add to this solution IMMEDIATELY

8 Feb.

Hybridization of - BM. Dex. from reconstituted mice



20. Mar. 85

Harvest of Karl Heng -NP - X63 transformant.

Cells

K_{TNP}-X63 ① (in 0.5 mg. G418) $4.4 \times 10^7 \times 1$

H_{TNP}-X63 ④ " $3 \times 10^7 \times 2$

1 April

Gene transfer to Born marrow cells

Vector pRHL

Protoplast. 20 min incubation

Reciepient cells CBA/HT6
- Dextercultur 1 Flask 25/2
- Fresh BM. 2 heads

Dextercultur incubation ō. Collagenase. 15 min
1.5×10^7 cells / total

Fresh born marrow Femur and Tibia
3×10^7 / total

Use Whole cells to fusion ~~Per~~ Protoplast.

fusion 40% PEG. 20 sec
5 µg poly-L-L Arg

FCS dilute
↓

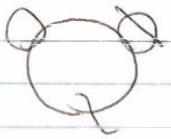
Yield
Dex 4×10^6 → 3 head /
Fresh-B.M. 8×10^6 → 3 head of CBA/J

CBA/J reciepient ~~90~~ 950R radiated.

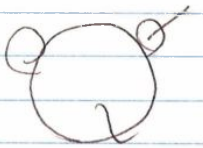
5×10^5 / head

1 April

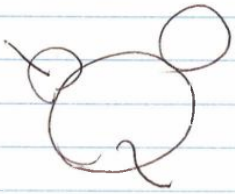
Four



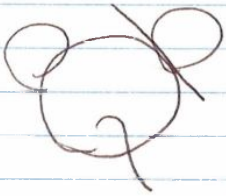
Med. Control



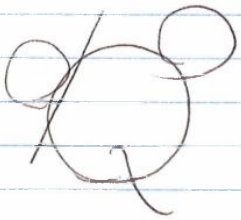
Fresh B.M. Control



Dex. Control



Dex. H-L



BM H-L

April 8 83'

Gene transfer to mouse and human born marrow cells

Mouse CBA/HIT6 { Jan. 3 8 2heads
Dex T6 25/Feb

mouse BM 3.5×10^7 total cells

human born marrow cells

2×10^7 total for the first fusion

fusion process → many clumps

↓ yield
2×10^6 in mouse

2×10^6 in human cells.

human born marrow cells
4×10^7 second time.

→ mix

2×10^7
for nude mice
950 rad Irradiated

2×10^7 ↔ fusion
↓
2×10^6 Yield.

divide to to tube

One for only add'n Chamber

2 1580

Yield of Cells after 12 day

gene transfered	Fresh bone marrow	→	→ BM (3heads)	1.06×10^7 /total	6
	"		→ Spl (3heads)	4.8×10^8 /total	22 ~~78~~
control	" (control)		→ BM (1heads)	3.7×10^6 /total	2
	" (")		→ Spl	1.04×10^8 /total	24
gene transfered	Dexter	→	→ BM (3heads)	2×10^5 total	1
			→ Spl (")	1.05×10^7 total	6
control	Dexter	→	→ BM (1heads)	2×10^5 total	1
			→ Spl (")	1.15×10^7 total	6

15. April 83.

Gene transfer to human born marrow cells

human born marrow cells
: 7.8×10^8 total

after fusion
Cells. $80 \times 4 \times 10^6$/ml 5ml
3.2×10^6 : 1.6×10^7 total
↓
3 capsules.

950 R.

26. April One capsule → split to new antic
6×10^6 cells $\times 2.5 \times 10^6$ cells

25 April One capsule → fusion.
2.5×10^4

20. Aug 83'

Hybridyation of 0.5 mg/ml Neo resistant surviving cells.

$\bar{c}$ ×63

				×63
Cells	FBM → BM	2×10^4	100	2×10^6
	FBM → Spl	1.6×10^5	50	8×10^6
	FBM → BM control	2.5×10^3		3×10^6
	FBM → Spl con	6×10^4	100	8.5×10^6

×63 Viable 95% <

total 1.6×10^7

Harvest the cells from B.M. reconstituted cells.

CBA/J. 3heads for gene transfered T6BM

1head for non ——— "

FBM→Spl $200 \times 4 \times 10^6$/ml 10ml

total 8×10^7 36well

control Spl $225 \times 4 \times 10^4$/ml 5ml

4.5×10^7 24well

BM 86×10^4/ml 5ml

4.3×10^6

BM control 30×10^4/ml 5ml

1.5×10^6

Gene transfer to mouse FBM : 26. April 85

Mouse CBA/HTG 2heads ♀

Born Mar.-1 1983

Cell No.

$1.66 \times 4 \times 10^4$ /ml 9.5ml

$\fallingdotseq 6 \times 10^2$ total.

0.5ml for Control.

$\therefore 5.5 \times 10^2$ for protoplast fusion.

after fusion

4.4×10^6 total.

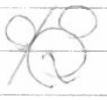
5×10^5 /head -4heads

Harvest of invitro selected cells

FBM → in vivo → Spl 6days

Cells 3.7×10^5 → 1. anemia

gene transfered 4heads



mom —— 1head

Med ctr 1

in vitro selected 1

27 April . 83

Harvesting cells and Fusion C-1 X63

F.BM. → spl → April 21 (selection started 0.8 mg Mec)

30×10^6/ml 3 ml 1×10^6 cell

35×10^6/ml 10 ml 35×10^6

for April 21

Gene

2.5×10^4 cells harvested from Dex → 10 days

2.5×10^8 cells from Gene non transfected cells

3. May 83

Gene transfer to Born marrow cells.
(mouse CBA MT6 1 head)

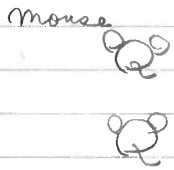
Cells 3.0×10^7 total
fusion 25 sec.

$3.0 \times 10^7 \rightarrow 0.5 \times 10^7$ for control

$\therefore 2.5 \times 10^7$ cells
Protoplast fusion
↓

Control for protoplast contamination (1/10 of plotoplast. $\downarrow 5 \times 10^5$ cell

after fusion
5×10^6 total
0.1ml for 1 head.



Gene transfered 3heads

4 May 85'

~~Titration of~~ Titration of and measurement of Culture soup of Transformants of BM-x63 Hybrids. anti-TNP antibody activity in

~~Transformed~~ wells
Hybridoma 2 x 2 - 48 wells

titration

Sp603
XR19
XR33
IR44
IgM10

8. May 83

Cell harvest from reconstituted mix

Jere transfered from B.M. reconstituted mix

Jere transfered 2 heads

Control 1 head

		Jere ⊕	Control
Spl.	ⓐ	5×10^7 (grey ⊕)	6×10^7 grey ⊕
B.M.	ⓑ	1×10^6 grey ⊕	1.5×10^6 grey ⊕

1.8×10^5 of ⓐ and ⓑ

add ×25 ×63

fusion 30 sec { 10% DMSO, 40% PEG

∴ 2.5×10^6 total

after fusion ⓐ ×63 → 1.8×10^6 total

ⓑ ×63 → 2.25×10^6 total

each 400 well → 50 for HAT Sp ⊕

50 for Neo selection

∴ 1.8×10^6 / well

2.25×10^4 / well

12 May. 83'

Harvest ~~of~~ the cell from 4 wells

May 8 → Spl Gene + 7×10^5/ml 2.9 ml
original Number 1.4×10^6/well
$\frac{2 \times 10^5}{5.6 \times 10^6}$ → × 28 ml concentrated?

May. 8 → Spl Gene − 5×10^5/ml 1.4 ml
original 2.4×10^6/well
$\frac{2 \times 10^5}{96 \times 10^5}$ → ×13~14 concentrated

×63 ~~→~~ 2.0×10^6/ml ~100 % Viable

Hybridization

1×10^8 Spl cells × 2.5×10^6 × 63

40 % PEG 10 % DMSO
30 sec.

Distribute to { 72 wells for Neo
72 wells for HAT

Control 1/2 of cells 24 wells for HAT
24 wells for Neo

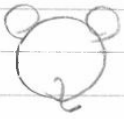
Control of 0.5 mg Neo effect

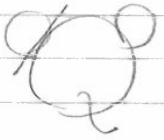
1×10^4 cell → resuspend to 0.5 mg neo
8 wells

16-1 May. 87.

Harvest of cells from Gene transfered BM. reconstituted mice.

Mice CBA/J. ← CBA TT6

a  Gene transfered B.M.

b  Fresh B.M. + protoplast.

a. born marrow 3.8×10^6 total

Spleen 1×10^8 total

b. born marrow 3.2×10^6

Spleen 3.4×10^7 total

16 May 83

Hybridization — 1 day in vitro maintained

×63 ↔ BM+protoplasts reconstituted mice Spl cells

Cells { ×63 1.5 × 10^6 ; BM+protoplasts 5.0 × 10^5

30 sec at in { 40 % PEG (BDH) ; 10 % DMSO (

19 May 83

Fusion. c. BM. gene transfer ⊕ BM → BM.

9 × 10^4 cells after 4 days selection.

50 times of ×63.

{ 48 well ×/ea ; 48 well HAT

20 May 83

Hybridoma preparation

Cells ① G(-) → Spl → 5 day selected

from 4 wells ~~2.5 x 10^5~~ 2 x 10^5

Cells ~~2.5 x 10^5 x~~ x 63 cells

after fusion 1 x 10^6 cells : 1/5

② Protoplasto + BM → Spl → 4 day select

from 4 wells

7.5 x 10^4

25 times cells

↓

2.1 x 10^5 cells → 10 well (2 x well)

96 well plate x 10 → 5 New 1000 cells/well

→ 5 HAT

③ Protoplasto + BM → BM →

Thur June 4 83

Gene transfer to Bone marrow cells

Protoplasto preparation follow to the improved modified method by Bill.

Thursday CBA/J T6/T6

4.5×10^6/ml 1 ml

$\therefore \div 5 \times 10^7$ cells

~~5 x 10~~ 1×10^{6} for

after fusion

1.5×10^6/ml 5 ml

2.5×10^6

6. June 83'

normal BM reconstituted mouse

3. May →

Spleen normal size

Cells 1.2×10^8 total spl

3×10^8 ×63 cells

4×10^8 total BM

1×10^8 ×63 cells

Fusion { Spl 20 ~ 35 sec

BM 25 ~ 30 sec

X. June 83'

BM (Gene transfered)

26 April Spl normal size ~~all~~ a little bigger some small colonies

$250 \times 10^6 \times 10$

Cells { Spl 2.5×10^2 ?

B.M. 8×10^6 total

×63 1.25×10^8 total

1 : 3

↓ ↓

BM Spl.

20 June 83

Hybridization.

X63 ↔ { Spl / BM — 17 days after reconstitution

Spl	5×10^7	→ X63	4×10^7	24×3 plate
BM	4×10^6	X63	1×10^7	24 wells

Hybridization 23 June 83'

{ Spl 8×10^7 / BM 1.5×10^7 — 20 days after

Gene transfer to B-1 cells 3. Aug

mouse CBA/HT6

Cell 3×10^7

after fusion 5×10^6

5×10^5 / heads 3 heads

Gene transfer BM.

CBA/J 3 heads.

CBA/HT6. 1 head (

$4 \times 10^7 \rightarrow 8 \times 10^6$.

20 %.

Small amounts of Protoplasts

Hybridization

Vector : PRHC

Spl × × 63 Mouse Aug. 3 reconstituted

BM

Spl 2.8×10^7 total → Spl RBC

BM 1×10^5 → BM

Spl cell : ×63 1 : 2 a 3

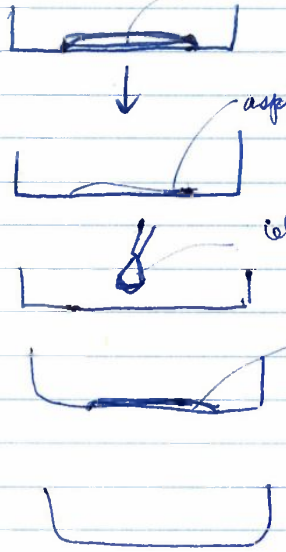
BM : ×63 1 : 10

Cells were resuspended in 2 mg/ml 10MT/S

Gene transfer to B.M.

2×10^2 20 µl pelet of Protoplasto
in ~~100~~ µl

H-2
~~30~~ % PEG ~~5.10 Poly L A 4~~ 2ml (A)



2 min

21 Aug.

Gene transfer

Vector pRHILTNP

Cell Spl (T6) $\{1\times10^8 \rightarrow 3\times10^7$ for use
B.M (T6) 3×10^7

Step . 0.1ml 33% PEG

0.1ml 3×10^7 cells + {5ul of cell Protoplast pellet}

0.1ml in H-21

add.

mixture (@ 0.84g + 0.2ml DMSO + 0.9ml H-21)

swelling gently. ~~for~~ 1min
stand at rt. 1min

dilute c H-1-21 10ml

37°c 5min

add DNAase Room temp. 10min

Spindown

↓

final cell No.

Spl 1×10^5 → 1head

B.M. 3×10^6 → 3heads ∴ 1×10^6/head

Gene transfer to BM cells

Step.

Sel cells
BM cells
↓
wash x2 c αMEM
↓
resuspend to 1ml.
αMEM 1μg/ml P-L-Arg
↓ 5 min
spindown
↓
resuspend 1ml of αMEM
add 2ml of
Protoplast in 1% Sucrose H21
↓
incubate 10 min
↓
Spindown.
↓add 0.7ml of
50% PEG.
15 sec.
↓
add 10ml of αMEM.
↓
distribute to 2 well plate

Positive control
x63

Strategy.

~~B-B~~ B.M.
↓
Gey's ~~stre~~ treatment
↓ wash ē 5% Sucrose H-21 Mg⊕

Protoplast.
↓
5% Sucrose.

↓ mix in 0.1ml 5% Sucrose B

~~40 %~~ A sol { 0.8ml PEG.
0.2ml DMSO.
0.8ml 10% Sucrose Mg

0.1ml make equal Sol

0.8ml of

0.1ml of A

2×10⁵/ml

2×10⁸ 40% 2×10⁸

max | 90 sec

4% PEG → 0
1% DMSO
5% Sucrose.

add { 5% Sucrose
Med. 0.4ml

1mg Neo

3 days 48h

3.75.

O H21. 5% Sucrose $MgCl_2$

H21 5% Sucrose 10% FCS 100 µg/ml Kanamycin
New - 0.5 ~ 1.0 mg/ml

B.M.

↓

wash x 2 DMEM

↓

resuspend to

5 µg P-L-Arg (1 ml)

↓ 5 min

tube wash ō 9 ml DMEM

↓ resuspend to 1 ml

↓ add 2 ml of

10 min at r.t.

↓ spin down

~~add~~ resuspend to 0.1 ml

50 % PEG 1.0 ml

15 sec

↓ 10 ml of DMEM

Protoplast

↓

resuspend to 1% Sucrose

10 ml

B.M.

↓

5 µg/ml 10 µg/ml p-L-Arg 3 min

↓

protoplast

↓ 10 min

resuspend to 0.1 ml

0.6 ml PEG

plate 15 sec

add FCS.

B.M. cells

wash x 2

↓

B.M.

wash x 2

↓

(P-L-Arg) 5 µg/ml 3 ml 5 min on ice

↓ wash = MEM.

mix ≈ E. coli → 1 ml 2 ml

10 min at r.t.

↓ wash x 1

resuspend to 0.1 ml

↓

0.9 ml of 50% PEG

↓ 15 sec

Stop ≈ FCS

19 Sept. 83

Protoplast. suspension. Buffer

0.6 M KCl – Tris-HCl

0.05 M tris-HCl 7.5

Fusion mixture

0.05 M tris + 10mM $CaCl_2$?

MW 74.56. 0.6 M. KCl

100 ml 0.6.

74.56

6

44.736 4.47 g / 100 ml

~~44.736~~

Protocols 19 Sept. 83

Cells	Protoplasts	DMSO	PEG	second
Spl	2×10^7	10%	8000	20 sec
	2×10^8		45%	
BM	2×10^9			

Other factors

- 2-ME ?
- DMSO for recover ?
- feeder layer cells

19. Sept. 83'

Buffer for fusion:
{ 50 mM Tris-HCl pH 7.4
{ 0.15 M. KCl

PEG 40% DMSO 10% } r.t.
Buffer 50%

Good

~~Exp. 5~~
20 Sept.

5×10^5 cells → ~~adh~~ 250 R

Sept. 26 83

Conditions for B.M gene transfer

Protocol

B.M
2×10^6 / group.

Protoplasto (−).

① ~~Hcl~~ Buffer 50mM Tris-HCl 150mM KCl

② Buffer + Sucrose 1%

③ Buffer + 1mg/ml ATP(Na)

④ αMEM

⑤ Mixture ratio.

Buffer	0.9 ml
DMSO	0.2
PEG	1.0 g

→ 1 ml to → 0.1 ml Cell suspension

r.t. 30 sec.

Result

① > ② > ③ >> ④.

Sept. 27 83'

Conditions of B.M. gene transfer

- Buffer ~~PBS~~ NaPBS
- KPB + KCl
- dilute a FCS
- Original

Buffer Buffer Buffer

Buffer

KPB - KCl

- pH 7.2
- 0.01 M
- 0.15 M

45 % PEG

1 g PEG
0.2 ml ~~PBS~~ DMSO
0.8 ml

Vector pR_{HL} TNP. 20 Sept. 83

Reciepient cells. { Spleen
B.M.

Condition.

{ PEG 6000. 1 g.
DMSO. 0.2×2 ml
KPB + KCl 0.8 ml.

take 1ml and to the 0.1ml of B.M. cell + protoplast.

↓

20 sec.

add 10 ml FCS ← 37°C pre-warmed

Thy. → RNA
Protein

Spl. ~~B.M.~~ → RNA transient expression 48 h

→ Protein 48 h

→ Hybridization 5 days selection

BM → RNA. 3×10^7.

Protein 3×10^7 to 7.

in V.V.O.

~~in vitro~~

in vitro selection and proliferation → in V.V.O
→ Hybridize

28. Sept. 83

Fusion transfer in BM Spl Thy

Condition { PEG 6000 (Koch-Light) 1g
DMSO. 0.22ml
Tris-HCl - KCl buffer 0.8ml

20 sec

Result

Bad

May be Koch-light is toxic for BM.

But @ Thy's viability is good.

Thy > B Spl > BM.

B.M. for reconstitution 2 heads
5×10^5

3. Oct. 83'

Gene transfer to B.M (fused)

Protoplast pRHC TUP

fusion condition ~~x~~ 20 sec.

{ 45 % PEG (8000) Fisher
| Buffer 0.8 ml { Tris-HCl 50 mM, KCl 0.6 M.
| DMSO 10 %

Dilution c Tris-HCl 50 mM
KCl 600 mM

Viability after fusion

10 % FCS RPMI → 18 well of [illegible]

{ [illegible] cell → [illegible]
{ B.M → Fisher med 20 % Horse Serum
↓
distribution days
[illegible]
Dexta

25. Oct.

Fusion: ×63

12 oct. reconstituted ē. 1×10^5

cells

Spl 5×10^7 cell / T

B.M 2×10^7 cell / T

Fusion

↓

48 wells

$72 / 2 \times 10^7$

↓

Img Neo.

Reconstituted mouse: CBA/J

distributed on layer cells

on 72 wells

3×10^5 / well

↓

9. Nov. 83

@ 11. Nov.

almost 1 month.

Spl

6×10^7

↓

for TCGF cells

↓

24 wells

B.M.

3.7×10^7

↓ with altured line

6×10^6 / fl/psk / 6ml

2 flask.

↓

$2,5 \times 10^7$ cells

↓

Hybridoma ē. ×63

~~Dex~~ Dec 5. 83'

Gene transfer to Dexter cultured cells.

Vector pRHC ~~TNP~~

Cells CBA/J Dex Oct. 2 →
Cells 1.47 x 10^6/ml 8.5 ml

Protocol

Protoplast.
resuspend to appropriate Vol of 20% Sucrose ~~Hcl~~ Tris
↓
dilute x2 ~ 50mM Tris-HCl 0.6M KCl
↓
take 0.1ml

add to cell pelet (Dex)

Fusion mixture

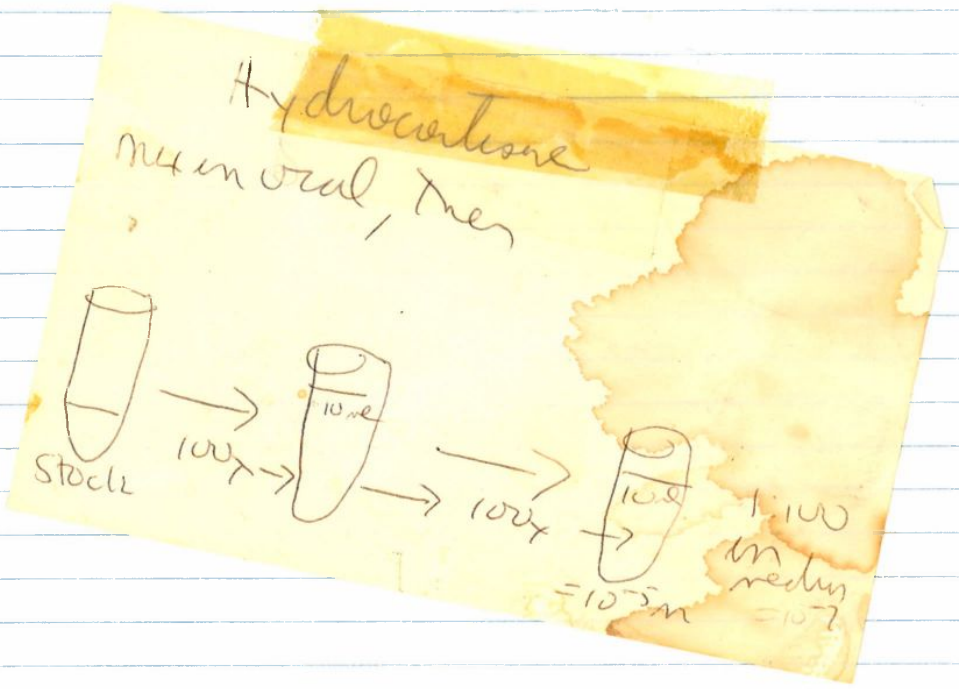
PEG 8000	0.8ml
DMSO	0.2
Tris-KCl	0.8 ml

↓ ← 37°C ~~20 sec~~

dilute ~~1~~ Tris KCl
↓
FCS layer 3. 10min
↓
Dex + 0.5mg Neo 10ml
↓
in 96well plate.

New method for stable protoplast

incubate for 30 min → spin down in (DNAse ① 10% Sucrose Tris HCl ② H-21)
↓
add 5ml of ② and spin at 5 3min
↓
Soup spin 3500 for 20 min
↓
resuspend in Tris HCl 20% Sucrose or 10% Sucrose
↓
add same amount of Tris - 0.6M KCl
for use


Hydrocortisone
NH4 in oral / Iner
10 ml
Stock
100x
100x
100x
1:100
in media
=10⁻⁵M
=10⁻⁷

23. Jan 84

Gene transfer to Reconstituted Spl cells
reconstituted ē Dexter cells

12d

5×10^7 from 1 spl

20 sec PEG 8000

in 0.5 mg Neo

27 Jan 84.

half →

48 well — ~~Meth~~: 1.5 ml 2.1% Mth - Cell-
0.5 @ HS
0.5 Dex 10 mg/ml Neo
0.3 ml Cond Med
50 µl / well

48 well
1.5 ml Dex
0.5 " Cond Med
0.5 10 mg/ml Neo

Whole cells → 5×10^4 ~~total~~ × 2 ml
$\therefore 1 \times 10^5$ cell
↓ in v:vo inject.

Gene transfer to the Reconstituted spl cells. 8 Feb

Cells: 3×10^6 total

PEG 8000 40%
DMSO 10%
Buffer
} → 1 ml 20 sec

0.75 ml Conditioned Med
0.75 ml Fresh Med
0.5 Met - B Cellulose
} → 4 wells

2 mg Neo

Mar. 7

Gene transfer to Dexo.

1×10^7 cells.

0.84 g PEG 8000
0.20 g
1.0 ml Buffer

→ 1 ml → 20 sec

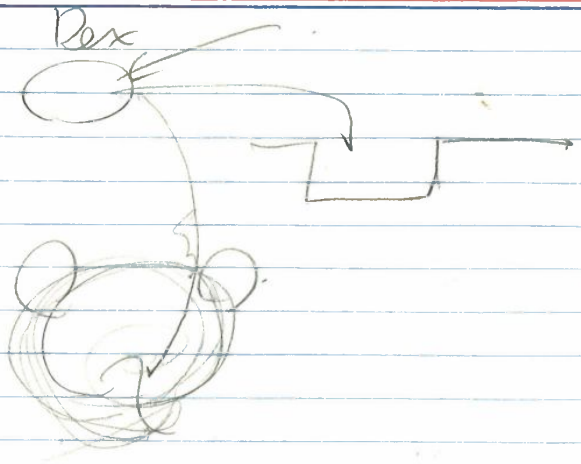
Cell Protoplast

→ Buffer ō 20% Sucrose 50 µl
Only the Buffer 50 µl

→ wash ō FCS layer
Pw 10 ml

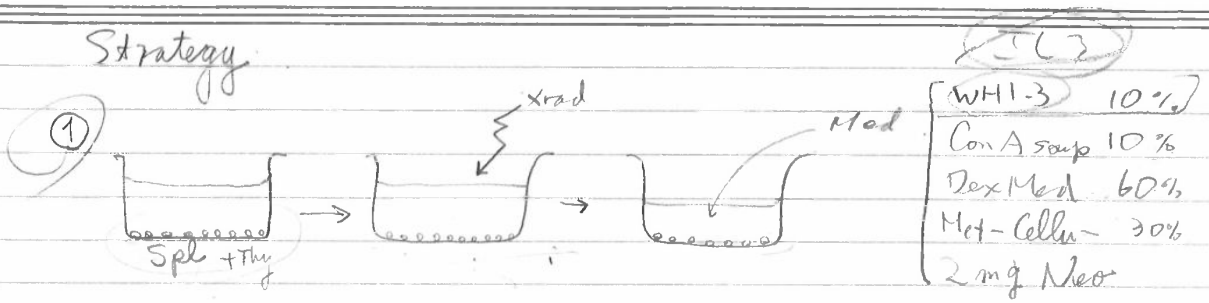
3 ml Con A supp. 10% FCS
2 ml Met-Cellulose
5 ml DexoMed.

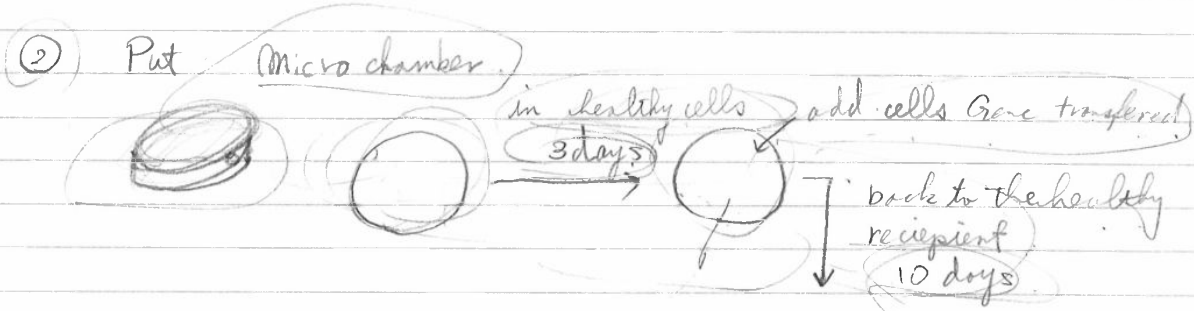
2 mg. Neo


Dex

28 : Mar

Strategy





Recipie for Collagenase.

150 mg Collagenase in 100 ml PBS.

28. June 84

RNA concentration check

Dex. → 5µl (reconstituted) 12 day.

	260	~~x 250~~	280	~~280~~
Control.	0.69		0.35	0.30
(1)	0.20		0.10	0.09
(2)	1.18		0.58	0.50
Dex 24 hr Apr.	0.48		024	0.20

DNA concentration of

Dex. → reconstituted 5µl

×200.

	230	260	280	
Dex con	0.03	0.065	0.035	0.65µg/µl
Dex 1	0.01	0.02	0.005	0.2µg/µl
Dex 2	0.03	0.07	0.03	0.7µg/µl
Dex P				

8. July

Southern

①	2M3HL4 2.2 µg/µl		20 µg 9 µl
②	Dex 1. 0.2 µg/µl	— 100 µg/µl	
③	Dex 2. 0.7 µg/µl	— 29 µl	
④	Dex Control 0.65 µg/µl	- 31 µl	

20 µg. BamHI

	DNA	BamHI	H_2O	Buffer
1	9 µl	2 µl	91 µl	11
2	100 µl	2 µl	0	11
3	29 µl	2 µl	70+1 µl	11
4	31 µl	2 µl	69 µl	11

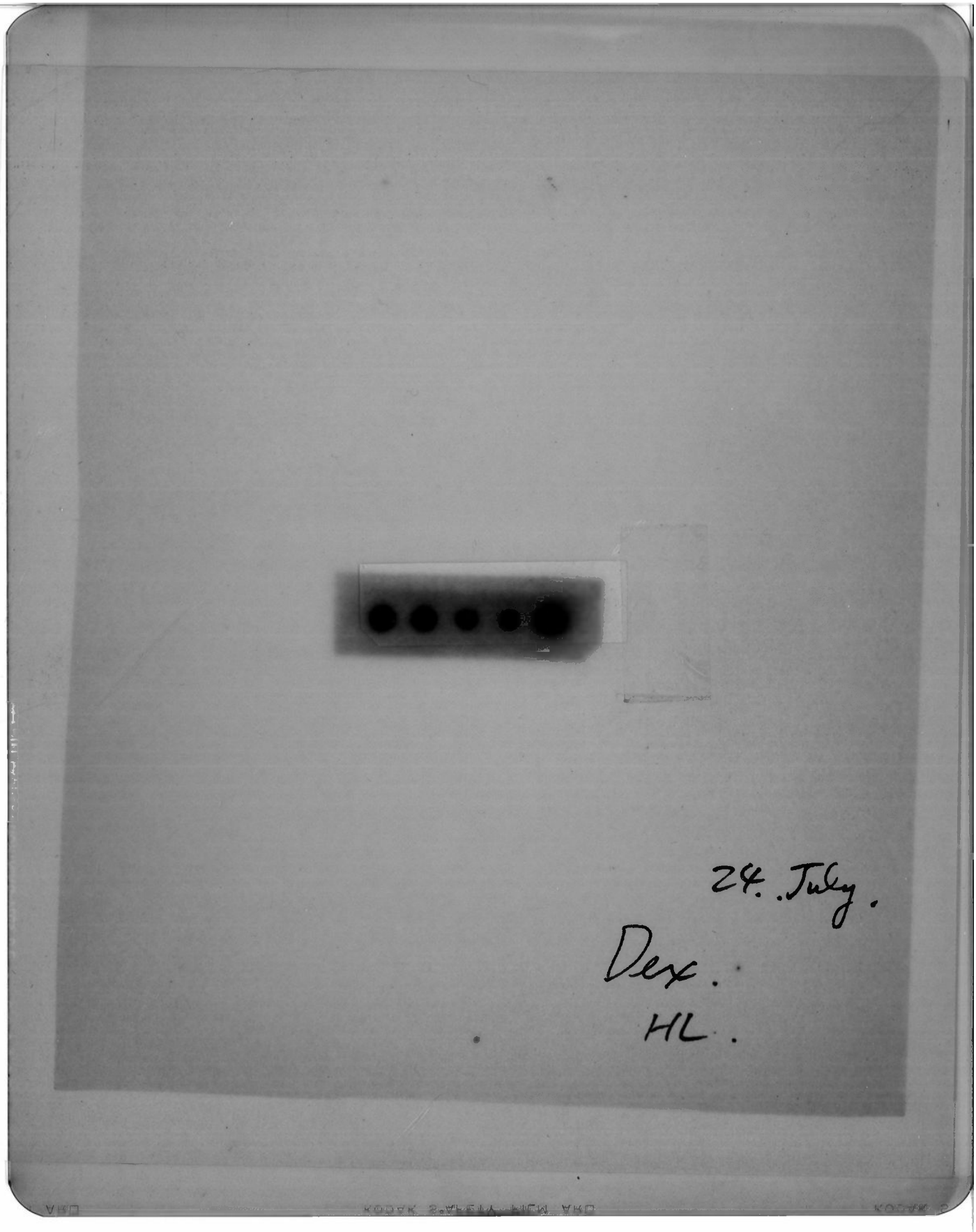
before transfer

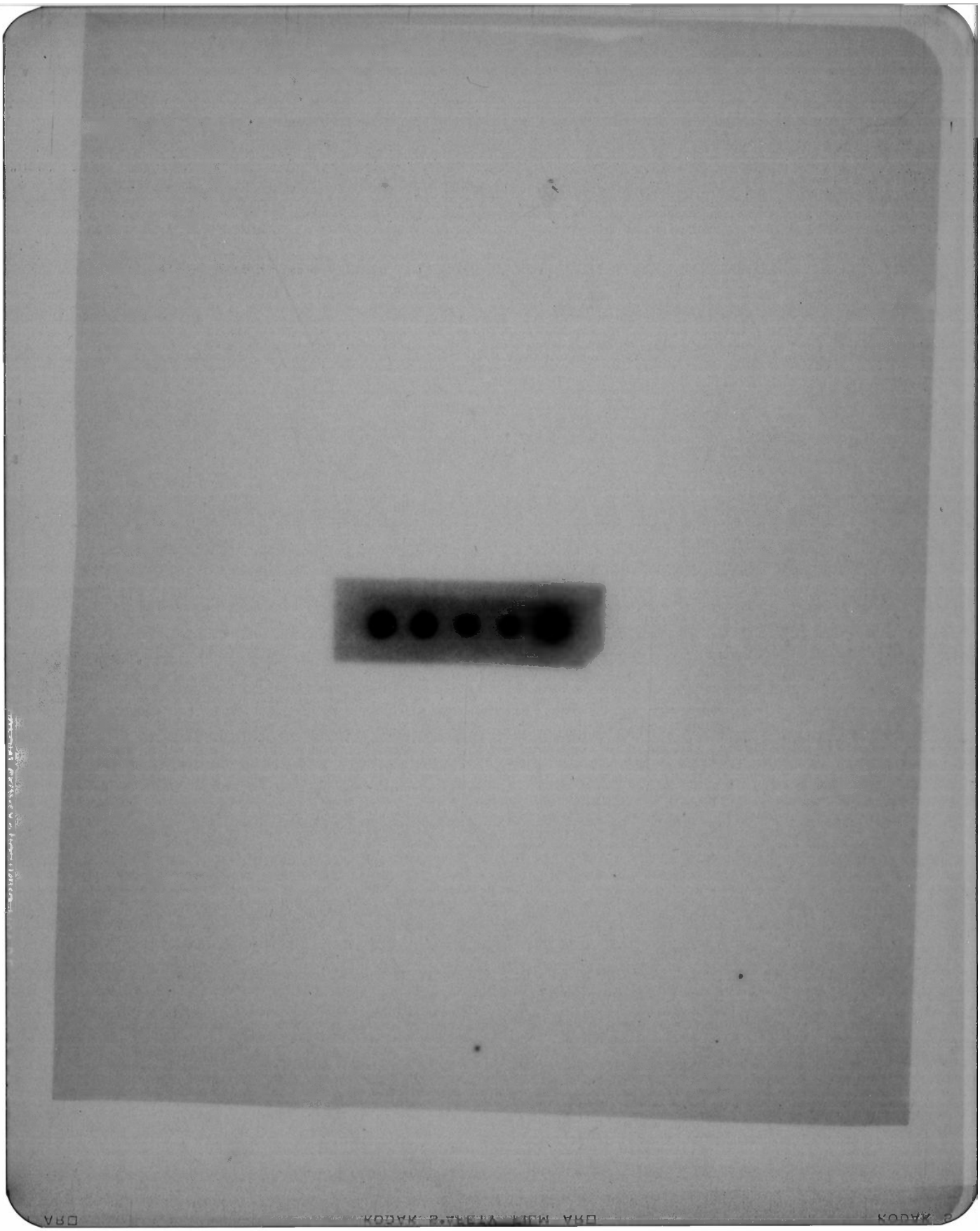
after transfer.

Gent transfer to Dex - Again /

Protoplast incubate for 40 min

1st wash.
3500 15 min
↓
add 6 ml
↓ Spin ò or ordinary small centrifuge
Soup
↓
2 add 6 ml of Buffer
↓
3500 15 min
↓
3 add 3 ml 20% Sucrose Med
+
9 ml Buffer
Spin down
↓
{ 0.84 g PEG
1 ml Buffer
+ poly-L-Arg
20 sec
↓
Add med
↓
FCS Gradient


24. July.
Dex.
HL.



24. July.

Dex.

HL.

Bactrim™ Roche®

Strong Sterile Co-trimoxazole Solution B.P.

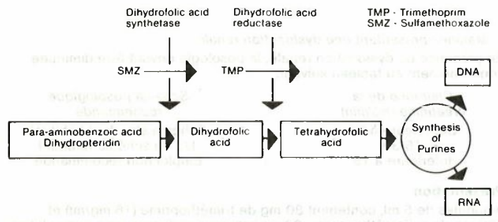
Antibacterial Agent

For **intravenous infusion** only

Action

'Bactrim' (trimethoprim and sulfamethoxazole) blocks two consecutive steps in the biosynthesis of nucleic acids and proteins essential to many bacteria (Figure 1). Sulfamethoxazole inhibits bacterial synthesis of dihydrofolic acid by competing with para-aminobenzoic acid. Trimethoprim blocks the production of tetrahydrofolic acid from dihydrofolic acid by reversibly inhibiting the required enzyme, dihydrofolate reductase.

FIGURE 1



The effect of the dual consecutive action is to reduce the minimum inhibitory concentration of each agent (synergism) and to convert a bacteriostatic action to a bactericidal action.

Indications

'Bactrim' (trimethoprim and sulfamethoxazole) is indicated in the treatment of the following infections when associated with the gram-positive and gram-negative organisms listed in Table I.

- Upper and lower respiratory tract infections (particularly chronic bronchitis and including acute and chronic otitis media).
- Acute, recurrent, and chronic urinary tract infections.
- Genital tract infections (uncomplicated gonococcal urethritis).
- Gastrointestinal tract infections.
- Skin and soft tissue infections.

'Bactrim' is also indicated in the treatment of *Pneumocystis carinii* pneumonitis.

Table I
List of susceptible organisms

Streptococcus pyogenes	Klebsiella spp.
Streptococcus viridans	*Enterobacter (Aerobacter) aerogenes*
Staphylococcus aureus	*Proteus mirabilis*
Staphylococcus albus	*Proteus vulgaris*
Diplococcus pneumoniae	*Salmonella typhi*
Haemophilus influenzae	*Salmonella paratyphi*
Neisseria gonorrheae	*Salmonella typhimurium*
Escherichia coli	*Salmonella enteritidis*
	Shigella spp.

'Bactrim' for Infusion is indicated in the treatment of serious systemic infections such as meningitis and septicemia caused by susceptible organisms as well as in the treatment of *Pneumocystis carinii* pneumonitis, when oral administration is not practical.

'Bactrim' is not indicated in infections associated with *Pseudomonas, Mycopiasma,* or viruses.

Contraindications

'Bactrim' is contraindicated in patients with evidence of known hypersensitivity to trimethoprim or sulfonamides, marked liver parenchymal damage, blood dyscrasias, or marked renal impairment when measurements of drug concentrations cannot be done (See Precautions).

'Bactrim' should not be given to premature neonates or infants less than two months of age (See Precautions). It is contraindicated in pregnancy and during the nursing period. If pregnancy cannot be excluded, the possible risks should be balanced against the expected therapeutic effect.

Precautions

General

As with other sulfonamide preparations, critical appraisal of benefit versus risk should be done in patients with liver or renal damage, urinary obstruction, blood dyscrasias, allergies or bronchial asthma. The possibility of superinfection with a non-sensitive organism should be borne in mind.

If 'Bactrim' for Infusion is administered on an emergency basis to neonatal infants, a potential complication in this age group is kernicterus.

In patients with renal impairment, a reduced or less frequent dosage is recommended in order to avoid accumulation of trimethoprim and sulfamethoxazole in the blood. For such patients, serum drug concentration measurements are necessary. 'Bactrim' should not be used when the creatinine serum concentration is above 2 mg/dl in order to avoid possible permanent impairment of renal function. Because of possible interference with folate metabolism, regular blood counts are advisable in patients on long-term therapy, in those who are predisposed to folate deficiency (i.e., the elderly, chronic alcoholics and rheumatoid arthritis), in malabsorption syndromes, in malnutrition states, or during the treatment of epilepsy with anticonvulsant drugs such as phenytoin, primidone or barbiturates. Changes indicative of folic acid impairment have, in certain specific situations, been reversed by folinic acid therapy.

Drug Interactions

PABA or its derivatives antagonize sulfamethoxazole. Increased sulfamethoxazole blood concentrations may occur in patients who are also receiving urinary acidifiers, oral anticoagulants or sulfonylurea hypoglycemics, phenylbutazone, oxyphenbutazone, indomethacin, sulfinpyrazone or salicylates.

Adverse Reactions

Hematological changes have been observed in some patients, particularly the elderly. The great majority of these changes were mild, asymptomatic, and proved reversible on withdrawal of the drug. The reported changes were primarily neutropenia and thrombocytopenia. Those observed less frequently include: leukopenia, aplastic and hemolytic anemia, purpura, agranulocytosis and bone marrow depression.

Other side-effects which have been observed most frequentiy to date include: nausea, vomiting, gastric intolerance and skin rash. Those of less frequent occurrence include: diarrhea, constipation, flatulence, anorexia, pyrosis, gastritis, gastroenteritis, urticaria, headache and liver changes (as indicated by abnormal elevations in alkaline phosphatase and serum transaminase levels). There has also been an occasional report of the following side-effects: erythema, edema, pruritus, toxicoderma, photosensitivity, glossitis, stomatitis, dyspepsia, dry mouth, dysuria, oliguria, anuria, hematuria, urgency, dyspnea, tremor, vertigo, tiredness, jaundice, vision troubles, drug fever, alopecia, epistaxis, black tongue, kidney changes (as indicated by abnormal elevations in blood urea nitrogen, blood non-protein nitrogen, serum creatinine, and urine protein levels) and anaphylactoid reactions (sweating and collapse). Goiter, diuresis and hypoglycemia have occurred rarely in patients receiving sulfonamides.

Treatment of Overdosage

If poisoning occurs from the ingestion of an overdosage of 'Bactrim', remove the agent from the stomach by lavage and/or emesis. If renal function is normal, force fluids orally or parenterally to promote excretion. Since both components are readily dialysable, dialysis should be considered in patients with impaired renal function.

There is no known antidote for sulfonamide poisoning, however, calcium folinate (3 to 6 mg I.M. for 5 to 7 days) is an effective antidote for trimethoprim.

There has been no experience with deliberate or inadvertent overdosage in humans with 'Bactrim' for Infusion. When repeated doses are required, large volumes of infusion solution may induce fluid overload. Appropriate therapy, *e.g.,* diuretics, may be required.

Dosage and Administration

I. intravenous Administration

'Bactrim' for Infusion may be used in patients who cannot take oral medication or who need rapid attainment of high serums concentrations.

PARENTERAL TREATMENT SHOULD BE TERMINATED AND ORAL TREATMENT INSTITUTED AS SOON AS POSSIBLE.

Method of Dilution

CAUTION — 'BACTRIM' FOR INFUSION MUST BE DILUTED IN STERILE 5% DEXTROSE IN WATER OR RINGER'S SOLUTION OR SODIUM CHLORIDE 0.9% SOLUTION PRIOR TO INFUSION ADMINISTRATION. DIRECT INTRAVENOUS INJECTION IS NOT RECOMMENDED. DO NOT MIX THE PREPARED INFUSION SOLUTION WITH OTHER DRUGS OR SOLUTIONS.

Each 5 ml ampoule of 'Bactrim' for Infusion should be diluted with 125 ml of 5% dextrose in water or Ringer's solution or sodium chloride 0.9% solution. The prepared solution must be kept at room temperature (15-30°C) and administration started within 1 hour.

NOTE: In those instances where fluid restriction is desirable, each ampoule may be added to 75 ml of 5% dextrose in water or Ringer's solution or sodium chloride 0.9% solution. Under these circumstances the solution should be mixed just prior to use and administration completed within 1 hour.

If upon visual inspection there is cloudiness or evidence of precipitation after mixing, the solution should be discarded and a fresh solution prepared.

a) *Pneumocystis Carinii Pneumonitis:*

Children and Adults:

The recommended daily intravenous dosage is 20 mg trimethoprim/kg body weight + 100 mg sulfamethoxazole/kg body weight. This daily dosage is to be divided into four equal doses infused over a period of 1/2 to 1 hour, at 6 hour intervals, until oral therapy can be instituted.

Volume of undiluted 'Bactrim' for Infusion per body weight*
(conversion factor 1.25 ml/kg)

body weight (kg)	Volume of undiluted 'Bactrim' for Infusion (ml)	
	Total Daily Dose	Doses Every 6 hours (q.i.d.)
5	6.3	1.6
10	12.5	3.1
20	25.0	6.3
40	50.0	12.5
60	75.0	18.8
80	100.0	25.0

*'Bactrim' for Infusion must be properly diluted (see Method of Dilution) and administered at 6 hour intervals.

b) *Serious Systemic Infections:*

Aduits: The intravenous dosage of 'Bactrim' for Infusion depends on the severity of the infection. A dose of 160 to 240 mg trimethoprim + 800 to 1200 mg sulfamethoxazole (10 to 15 ml of undiluted 'Bactrim' for Infusion) may be given every 6, 8 or 12 hours. The dosage must be properly diluted (see Method of Dilution) and infused over a period of 1/2 to 1 hour.

Children: The recommended daily dosage for children is 5 to 10 mg trimethoprim/kg body weight and 25 to 50 mg sulfamethoxazole/kg body weight. This daily dosage is to be properly diluted and administered in equally divided doses by Infusion over a period of 1/2 to 1 hour.

Volume of undiluted 'Bactrim' for Infusion per body weight*
(conversion factor 0.31 to 0.63 ml/kg)

body weight (kg)	Volume of undiluted 'Bactrim' for Infusion (ml)			
	Total Daily Dose	Doses every 12 hours (b.i.d.)	8 hours (t.i.d.)	6 hours (q.i.d.)
5	1.6-3.2	0.8-1.6	0.5-1.1	0.4-0.8
10	3.1-6.3	1.6-3.2	1.0-2.1	0.8-1.6
20	6.2-12.6	3.1-6.3	2.1-4.2	1.6-3.2
40	12.4-25.2	6.2-12.6	4.1-8.4	3.1-6.3
60	18.6-37.8	9.3-18.9	6.2-12.6	4.7-9.5

*'Bactrim' for Infusion must be properly diluted (see Method of Dilution) and administered in equally divided doses.

II. For Patients with Impaired Renal Function:

When renal function is impaired, a reduced dosage should be employed using the following table:

Creatinine Clearance (ml/min)		*Recommended Dose Regimen*
Above 30	:	Usual standard regimen
15–30	:	1/2 the usual regimen
Below 15	:	Use not recommended

How Supplied:

5 ml ampoules containing 80 mg trimethoprim (16 mg/ml) and 400 mg sulfamethoxazole (80 mg/ml) for infusion with sterile: 5% dextrose in water or Ringer's solution or sodium chloride 0.9% solution.
Pack of 5 x 5.

Also available as:

'Bactrim' 'Roche' Tablets	Each containing 80 mg trimethoprim and 400 mg sulfamethoxazole. Bottles of 100 and 500. Unit dose blisters of 100.
'Bactrim' 'Roche' DS Tablets	Each containing 160 mg trimethoprim and 800 mg sulfamethoxazole. Bottles of 100 and 250.
'Bactrim' 'Roche' Pediatric Tablets	Each containing 20 mg trimethoprim and 100 mg sulfamethoxazole. Bottles of 100.
'Bactrim' 'Roche' Pediatric Suspension	Each teaspoonful (5 ml) containing 40 mg trimethoprim and 200 mg sulfamethoxazole; cherry flavoured. Bottles of 100 ml and 400 ml.

3411-02

This package insert issued December 1981, reprinted January, 1982.

ROCHE®

Hoffmann-La Roche Limited
Vaudreuil, Québec J7V 6B3

Bactrim™ Roche®

Strong Sterile Co-trimoxazole Solution B.P.

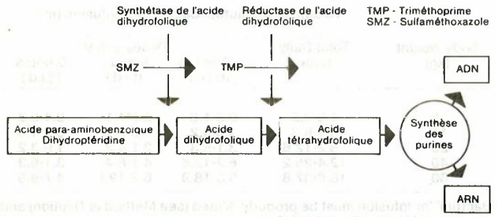
Antibactérien

Pour **perfusion intraveineuse** seulement

Effets

'Bactrim' (triméthoprime et sulfaméthoxazole) bloque deux étapes consécutives de la biosynthèse des acides nucléiques et de protéines essentielles à de nombreuses bactéries (Figure 1). Le sulfaméthoxazole inhibe la synthèse bactérienne de l'acide dihydrofolique par compétition avec l'acide para-aminobenzoïque. Le triméthoprime bloque la formation d'acide tétrahydrofolique à partir de l'acide dihydrofolique en inhibant de façon réversible l'enzyme nécessaire, la réductase de l'acide dihydrofolique.

FIGURE 1



Cette double action consécutive a pour effet de réduire la concentration inhibitrice minimale de chaque agent (synergie) et de transformer une action bactériostatique en action bactéricide.

Indications

'Bactrim' (triméthoprime et sulfaméthoxazole) est indiqué dans le traitement des infections suivantes lorsqu'elles sont causées par les microorganismes gram-positifs et gram-négatifs énumérés dans le Tableau 1:

- Infections des voies respiratoires supérieures et inférieures (bronchite chronique, en particulier), y compris l'otite moyenne aiguë et chronique.
- Infections des voies urinaires aiguës, récidivantes et chroniques.
- Infections des voies génitales (urétrite gonococcique sans complications).
- Infections des voies gastro-intestinales.
- Infections de la peau et des tissus mous.

'Bactrim' est aussi indiqué dans le traitement de la pneumonite à *Pneumocystis carinii.*

Tableau 1
Liste des microorganismes sensibles

Streptococcus pyogenes	Klebsiella (toutes les espèces)
Streptococcus viridans	*Enterobacter (Aerobacter) aerogenes*
Staphylococcus aureus	*Proteus mirabilis*
Staphylococcus albus	*Proteus vulgaris*
Diplococcus pneumoniae	*Salmonella typhi*
Haemophilus influenzae	*Salmonella paratyphi*
Neisseria gonorrhoeae	*Salmonella typhimurium*
Escherichia coli	*Salmonella enteritidis*
	Shigella (toutes les espèces)

La solution 'Bactrim' pour perfusion est indiquée dans le traitement des infections générales graves comme la méningite et la septicémie causées par des microorganismes sensibles ainsi que dans le traitement de la pneumonite à *Pneumocystis carinii,* lorsque l'administration orale est peu pratique.

'Bactrim' n'est pas indiqué dans le traitement des infections à *Pseudomonas* ou à *Mycoplasma,* ni dans le traitement des infections virales.

Contre-indications

'Bactrim' est contre-indiqué en présence d'hypersensibilité connue au triméthoprime ou aux sulfamides, de lésions hépatiques graves, de dyscrasies sanguines ou de dysfonctions rénales graves lorsqu'il est impossible de déterminer les concentrations médicamenteuses (voir Précautions).

'Bactrim' ne devrait pas être administré aux nourissons nés avant terme ou âgés de moins de deux mois (voir Précautions). Il est contre-indiqué durant la grossesse et la période d'allaitement. Si la possibilité d'une grossesse ne peut être exclue, mettre en balance les risques possibles et l'effet thérapeutique recherché.

Précautions

Générales

Comme pour les autres sulfamides, il faut mettre soigneusement en balance les avantages prévus et les risques possibles chez les malades présentant une lésion hépatique ou rénale, une obstruction urinaire, des dyscrasies sanguines, des allergies ou de l'asthme bronchique. La possibilité de surinfection par un microorganisme non sensible devrait être prise en considération.

Chez les nouveau-nés, l'ictère nucléaire est une complication possible après l'administration d'urgence de la solution 'Bactrim' pour perfusion.

En présence de dysfonction rénale, on recommande de réduire la posologie ou d'espacer les prises pour éviter l'accumulation de triméthoprime et de sulfaméthoxazole dans le sang. Des déterminations de la concentration médicamenteuse sérique s'imposent dans ces cas. On devrait éviter l'emploi de 'Bactrim' lorsque le taux de créatinine sérique est supérieur à 2 mg/dl pour éviter le risque d'une atteinte permanente de la fonction rénale. Comme le médicament peut entraver le métabolisme des folates, on recommande des hémogrammes périodiques pendant un traitement prolongé, chez les malades prédisposés à une carence en folates (c'est-à-dire les sujets âgés, les alcooliques chroniques et les malades atteints de polyarthrite rhumatoïde), chez les sujets souffrant de syndromes de malabsorption ou de malnutrition, ou chez les épileptiques traités par des anticonvulsivants comme la phénytoïne, la primidone ou les barbituriques. Dans certains cas, les signes d'un trouble du métabolisme de l'acide folique ont été corrigés par l'administration d'acide folinique.

Interactions médicamenteuses

Le PABA ou ses dérivés sont des antagonistes du sulfaméthoxazole. Une hausse des concentrations sanguines de sulfaméthoxazole peut survenir chez les malades déjà sous traitement par l'un ou l'autre des agents suivants: acidifiants urinaires, anticoagulants oraux ou hypoglycémiants sulfonylurétiques, phénylbutazone, oxyphenbutazone, indométhacine, sulfinpyrazone ou salicylates.

Réactions indésirables

Des altérations hématologiques ont été observées chez certains malades, surtout des sujets âgés. La plupart étaient banales, asymptomatiques et ont été corrigées par l'arrêt du traitement. Les principales altérations signalées étaient la neutropénie et la thrombocytopénie. La leucopénie, l'anémie aplasique ou hémolytique, le purpura, l'agranulocytose et l'hypoplasie médullaire ont été observés plus rarement.

Parmi les réactions indésirables rapportées le plus souvent jusqu'ici, on remarque: nausées, vomissements, intolérance gastrique et éruption cutanée. D'autres, moins fréquentes, incluent: diarrhée, constipation, flatulence, anorexie, pyrosis, gastrite, gastro-entérite, urticaire, céphalée et aitérations hépatiques (mises en évidence par une élévation anormale de la phosphatase alcaline et des transaminases sériques). Ont également été signalés à l'occasion: érythème, oedème, prurit, toxicodermie, photosensibilité, glossite, stomatite, dyspepsie, xérostomie, dysurie, oligurie, anurie, hématurie, besoins impérieux, dyspnée, tremblements, vertige, lassitude, ictère, troubles de la vision, fièvre médicamenteuse, alopécie, épistaxis, glossophytie, altérations rénales (mises en évidence par une élévation anormale de l'azote uréique sanguin, de l'azote non protéique du sang, de la créatinine sérique et des taux de protéines urinaires) et réactions anaphylactoïdes (diaphorèse et syncope). En de rares occasions, goitre, diurèse et hypoglycémie sont survenus chez des malades recevant des sulfamides.

Traitement du surdosage

En cas d'intoxication à la suite de l'ingestion d'une dose excessive de 'Bactrim', retirer le médicament de l'estomac par un lavage gastrique ou des vomissements. Si la fonction rénale est normale, faciliter l'excrétion par l'administration forcée de liquides par voie orale ou parentérale. Comme les deux composants de ce médicament se dialysent facilement, songer à la dialyse chez les malades présentant une dysfonction rénale.

On ne connaît pas d'antidote aux sulfamides, mais le folinate de calcium (3 à 6 mg I.M. pendant 5 à 7 jours) est un antidote efficace du triméthoprime.

Aucun cas de surdosage volontaire ou accidentel par la solution 'Bactrim' pour perfusion n'a été rapporté chez l'homme. Lorsqu'il faut répéter les doses, il est possible que les larges volumes de la solution de perfusion entraînent une surcharge liquidienne. Un traitement approprié, p. ex. des diurétiques, pourra s'imposer.

Posologie et administration

I. Administration intraveineuse

La solution 'Bactrim' pour perfusion peut être administrée aux malades incapables d'ingérer des médicaments par voie orale ou chez qui il faut atteindre rapidement des concentrations sériques élevées.

LE TRAITEMENT PAR LA FORME PARENTÉRALE DEVRAIT PRENDRE FIN LE PLUS TÔT POSSIBLE POUR FAIRE PLACE AU TRAITEMENT ORAL.

Méthode de dilution

ATTENTION — LA SOLUTION 'BACTRIM' POUR PERFUSION DOIT ÊTRE DILUÉE DANS UNE SOLUTION AQUEUSE STÉRILE DE DEXTROSE À 5% OU DANS UNE SOLUTION DE RINGER OU DE CHLORURE DE SODIUM À 0.9% AVANT D'ÊTRE ADMINISTRÉE EN PERFUSION. L'INJECTION INTRAVEINEUSE DIRECTE N'EST PAS RECOMMANDÉE. UNE FOIS PRÉPARÉE, LA SOLUTION DE PERFUSION NE DOIT PAS ÊTRE MÉLANGÉE AVEC D'AUTRES MÉDICAMENTS OU SOLUTIONS.

Le contenu de chaque ampoule de 5 ml de solution 'Bactrim' pour perfusion devrait être dilué dans 125 ml d'une solution aqueuse de dextrose à 5% ou d'une solution de Ringer ou de chlorure de sodium à 0.9%. Une fois préparée, la solution doit être conservée à la température ambiante (15-30°C) et l'administration doit débuter dans l'heure qui suit.

NOTE: Lorsqu'il est souhaitable de limiter l'apport liquidien, le contenu de chaque ampoule pourra être dilué dans 75 ml d'une solution aqueuse de dextrose à 5% ou d'une solution de Ringer ou de chlorure de sodium à 0.9%. Dans ce cas, la solution devra être mélangée extemporanément et administrée au complet en moins d'une heure.

Si, une fois préparée, la solution présente des signes visibles de turbidité ou de précipitation, on devrait la jeter et préparer une solution fraîche.

a) *Pneumonite à Pneumocystis carinii:*

Enfants et adultes:

On recommande une posologie intraveineuse quotidienne de 20 mg de triméthoprime + 100 mg de sulfaméthoxazole par kg de poids corporel. Cette posologie quotidienne doit être fractionnée en quatre doses égales perfusées sur une période de 1/2 à 1 heure, à intervalles de 6 heures, jusqu'à ce qu'un traitement oral puisse être instauré.

Volume de solution 'Bactrim' pour perfusion non diluée en fonction du poids corporel*
(facteur de conversion 1.25 ml/kg)

Poids corporel (kg)	Volume de solution 'Bactrim' pour perfusion non diluée (ml) Dose quotidienne totale	Doses aux 6 heures (4 f.p.j.)
5	6.3	1.6
10	12.5	3.1
20	25.0	6.3
40	50.0	12.5
60	75.0	18.8
80	100.0	25.0

*La solution 'Bactrim' pour perfusion doit être diluée convenablement (voir Méthode de dilution) et administrée à intervalles de 6 heures.

b) *Infections générales graves:*

Adultes: La posologie intraveineuse de la solution 'Bactrim' pour perfusion dépend de la gravité de l'infection. On peut administrer une dose de 160 à 240 mg de triméthoprime + 800 à 1200 mg de sulfaméthoxazole (10 à 15 ml de solution 'Bactrim' pour perfusion non diluée) toutes les 6, 8 ou 12 heures. Cette posologie doit être convenablement diluée (voir Méthode de dilution) et la perfusion s'étendre sur une période de 1/2 à 1 heure.

Enfants: La posologie quotidienne recommandée pour les enfants est de 5 à 10 mg de triméthoprime et de 25 à 50 mg de sulfaméthoxazole par kg de poids corporel. Cette posologie quotidienne doit être diluée convenablement et administrée en doses fractionnées égales par perfusion sur une période de 1/2 à 1 heure.

Volume de solution 'Bactrim' pour perfusion non diluée en fonction du poids corporel*
(facteur de conversion 0.31 à 0.63 ml/kg)

Poids corporel (kg)	Volume de solution 'Bactrim' pour perfusion non diluée (ml) Dose quotidienne totale	12 heures (2 f.p.j.)	Doses aux 8 heures (3 f.p.j.)	6 heures (4 f.p.j.)
5	1.6-3.2	0.8-1.6	0.5-1.1	0.4-0.8
10	3.1-6.3	1.6-3.2	1.0-2.1	0.8-1.6
20	6.2-12.6	3.1-6.3	2.1-4.2	1.6-3.2
40	12.4-25.2	6.2-12.6	4.1-8.4	3.1-6.3
60	18.6-37.8	9.3-18.9	6.2-12.6	4.7-9.5

*La solution 'Bactrim' pour perfusion doit être diluée convenablement (voir Méthode de dilution) et administrée en doses fractionnées égales.

II. Malades présentant une dysfonction rénale:

En présence de dysfonction rénale, la posologie devrait être diminuée conformément au tableau suivant:

Clairance de la créatinine (ml/min)		*Schéma posologique recommandé*
Supérieure à 30	:	Schéma standard habituel
15-30	:	1/2 du schéma habituel
Inférieure à 15	:	Empioi non recommandé

Présentation

Ampoules de 5 ml, contenant 80 mg de triméthoprime (16 mg/ml) et 400 mg de sulfaméthoxazole (80 mg/ml) pour perfusion avec une solution aqueuse stérile de dextrose à 5%, ou une solution stérile de Ringer ou de chlorure de sodium à 0.9%.
Emballages de 5 x 5.

Également offert sous les formes suivantes:

Comprimés 'Bactrim' 'Roche'	Dosés à 80 mg de triméthoprime et 400 mg de sulfaméthoxazole. Flacons de 100 et 500. Doses unitaires sous plaquettes, emballages de 100.
Comprimés 'Bactrim' 'Roche' DS	Dosés à 160 mg de triméthoprime et 800 mg de sulfaméthoxazole. Flacons de 100 et 250.
Comprimés pédiatriques 'Bactrim' 'Roche'	Dosés à 20 mg de triméthoprime et 100 mg de sulfaméthoxazole. Flacons de 100.
Suspension pédiatrique 'Bactrim' 'Roche'	Chaque cuillerée (5 ml) contient 40 mg de triméthoprime et 200 mg de sulfaméthoxazole; aromatisée à la cerise. Flacons de 100 ml et 400 ml.

3411-02

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ROCHE ®

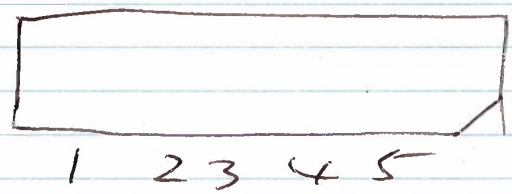
Hoffmann-La Roche Limitée
Vaudreuil, Québec J7V 6B3

19. July 84.

Dot Blot.

Dex (HL) → Spl. (BALB/J) →

1	Dex.	Control 28. June	6.9 µg	3 µl (20 µg)
2	Dex	Spl ①	20 µg	11 µl
3	Dex	HL Spl ②	11.8 µg	2 µl
4	Dex.	Spl. 24 May 84.	4.8 µg	4.3 µl
5	Spl 03			1 µl



Test of Antibiotics

{ Gentamycine ①
Tobramycine ②
Bactrim) 100 µg/ml

29. Aug. 84'

Gene transfer to Dex (Spl) WEHI III dependent cells

Protocol · 40~45 sec
50 % PEG 8000 in buffer
~~0.5 ml~~
ō ~~5×1~~ 5 µg/ml · Poly L-Lysin

PEG8000 1.0 g
Buffer 1.0 ml
poly-L-Lysin
→ dilute ō · α MEM → FCS (per

Med. for Dex ~~Med~~ cells

Dex med
Genta 100 µg/ml
Neo 0.5 mg/ml

for WEHI III cells

~~Dex~~ RPMI 1640 · 10 % FCS
Neo 0.5
WEHI 10 %

↓
0.5 mg Neo · 15 days
↓
1 mg Neo 11 days
↓
5×10⁶ inject to the CBA/J

12 Feb 85

Sol. for Protoplast washing 50 mg / 10 ml

~~0.6M Mannitol~~ 0.5 Mannitol - Buffer + Protease inhibitor

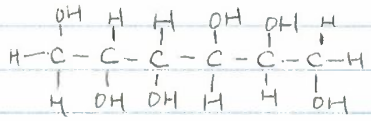
for cell - Buffer + 0.1 M Manitol

for cell fusion

- PEG 6000 40 %
- Buffer + 0.1 M mannitol 50 %
- Poly L.-L

30 sec

resolve in Buffer + 0.1 M ~~Mannitol~~ Mannitol



mannitol

15.1 g / l

20 11.2

50 ml of 0.5 M manitol Buffer 4.5 g

" 0.1 M " " 0.9 g D-M

50 mM Tris Buffer pH 8.0.

19+1 Feb 85

Sol for Protoplast wash

(XMEM (10% Sucrose 10 mM MgCl
↳ Spin
(resuspend to 0.6M Mannitol ~~1~~ 1ml
↳ ~~resuspend~~ dilute 9 ml of 0.1M Mannitol
buffer +50 µl of ×2 ~
↳ Spin ~~buffer~~
↓
Take soup as much as it possible

Will wash ~~d~~ c. 50mM tris pH 8.0 1KCl 0.5M

20 sec mix ↓

in Buffer + 0.6 Mannitol

24 Mar. 85'

Protoplast

lysozone double

incubat 30 min

add Sucrose Med
DNAase

↓

resuspend in 1ml Sucrose Med

↓

add 4ml 0.1 M Maitol Sol → 5 times diluted + protease inhi

↓ spin down.

↓

add cells in 0.1 M Maitol sol + protease inhibitor

↓

Spin down.

fusion { PEG 6000 1g
Buffer 1ml } 1ml

↓

20 sec → 60 sec gently
dilute in Buffer + protease inhibitor

↓

add → spin down.

↓ FCS gradient

3×10^6/ heads

2 heals % gene transfered
1 " normal BL1

26 Mar 85

new Buffer for Protoplast fusion

0.14M NaCl 20mM Tris-HCl 3mM KCl 0.8mM $MgSO_4$.

↑ ↑

5 M (1M sol) ×5 246.47

7,5 ml 5 ml 0,055g/250ml 0.05 g/250 ml

250 ml

Pansorbin (Calbiochem)

Protocol s
for the Preparation of B.M.

Wash c 20% Sucrose Buffer

↓ X 1

↓

≀

↓

DNAse 10 min

↓

Spin down

↓

resuspend 1ml of 10% Sucrose $MgCl_2$ sol

↓ spin down

Soup → dilute c. 0.1 M Manitol - Buffer { 0.15 M NaCl; 20mM Tris-HCl pH 7.4; 3mM KCl; 0.8mM $MgSO_4$

1ml 10% Sucrose → + 4 ml of ↗

↓

Spin down

↓

aspirate except 0.1 ml

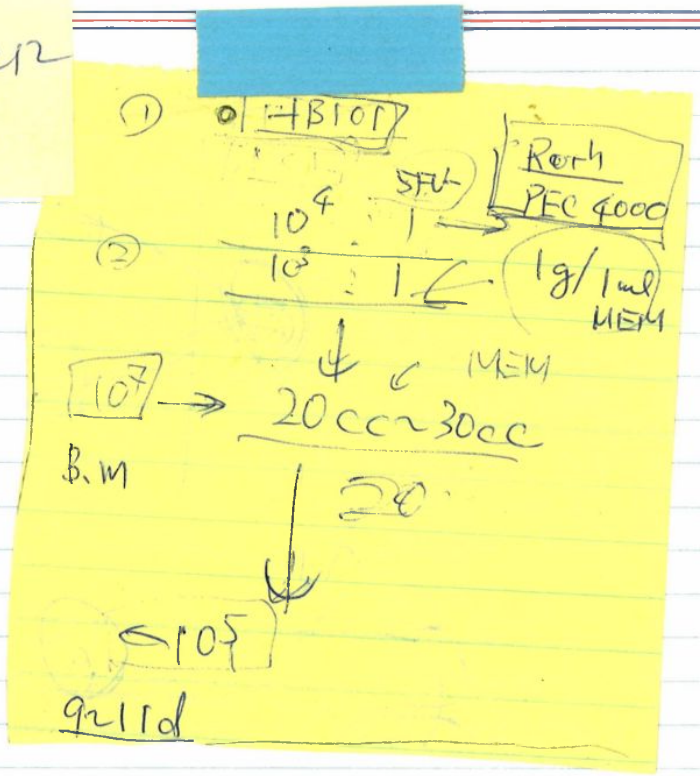
add one drop of 0.5 mg/ml χ^2-4 → take 0.05 ml → Mixture cell

cells wash c. 0.1 M Manitol

fusion Mixture { 1 g. PEG; 1 ml Buffer

Rescapin

212—212



1

30

1000/50 00

27 July

DNA digestion w Restriction Enzymes

		DNA		
BM	1	—		
	2	pSV2neo	RI	1x cell
	3	pSVHL	XhoI	
Spl	4	—		
	5	pSV2neo	RI	
	6	pRSVHL	XhoI	

50 µg DNA / mL : 0.5 mL each

DNA 50 µg

	DNA	Enz	10x Buffer	Enz 1500 µ/mL	H_2O
JP-2	50 µL	XhoI 25 µL	10 µL	200 µ/µL	25 µL
pSV2neo	30 µL	RI	10 µL	6 µ/µL	10 µL

2/ Aug. 85 -

Gene transfer to Bebbe B.M.

150 µg DNA / ml

0.5 ml.

	DNA
1.	—
2.	pSV2neo undigested
3.	pSV2neo . EcoRI
4.	JP2 Undigested.

204 19/118+19

3M XD/154

Sp603 90 C + ?

k.1 90 C +