LABORATORY NOTEBOOK

Tissue Culture 4/1/04

GENENTECH, INC.

NOTEBOOK NO. 11162
ISSUED TO John Ridgway
ON Feb 20 19 90
DEPARTMENT Cell Genetics 435
RETURNED 19

Witnessed by Rebecca Caspers
on March 1, 1991 pages #1-96

—SCIENTIFIC NOTEBOOK CO.—
2831 LAWRENCE AVE.
P.O. BOX 238
STEVENSVILLE, MI 49127
616-429-8285

Genentech Laboratory Notebook Procedures

Important steps for maintenance of your notebooks:

1) **Use dark permanent ink to record all of your work in your notebook on a daily basis. Sign and date each day's entries.**

2) **Permanently affix all attachments without covering any other entries. Please attach copies of any computer data entered into your computer files. These data are considered part of your experimental record.**

3) **Make any changes in data in dark permanent ink and, if appropriate, initial and date in the margin. Ensure that the original entry remains visible. Leave no open areas. When the book is completed line out any unused portion of a page at the end of each experiment.**

4) **Have a witness who understands and is aware of your work, but who is not directly involved in your project, sign and date your notebook entries (not later than one month after you do the work).**

5) **Don't hold back data for later entry and don't keep a "rough draft" notebook.**

6) **Report the quantitative or qualitative results only.** Avoid over broad and potentially inflammatory comments like "failed experiment", "doesn't work", or "toxic compound."

7) **The source and character of starting materials should be described.** Preferably, refer to the notebook pages describing the starting material and its method of preparation.

8) **Be sure your notebook record is understandable.** Omit abbreviations or slang that would not be understood by others working in your field. It is helpful to introduce each experiment with a statement of purpose, and make liberal use of cross-references to related experiments. While sufficient detail should be included to enable reproduction of experiments, it is acceptable to refer to conventional or published procedures. However, record any changes you may make to such procedures.

9) **Record your ideas too, not just experimental data.** This is important to demonstrate when an invention or thought occurred to you, and is an important part of establishing priority in inventorship contests. Don't hesitate to broaden the scope of your ideas; there is no reason to limit them to specific experiments planned for the next few days, although you should include as much detail as possible. Your notebook will not be published and there is no penalty for guessing wrong on notebook *idea* entries, so don't hesitate to let your imagination run.

10) **Try to keep a different notebook for each product and/or project. Please complete the table of contents indicating which product and/or project this work concerns.**

Genentech's success, and with it your own prosperity and research support, depends upon the quality and timing of your work. This may require that we prove what you did and when you did it in the face of a contrary challenge. Such challenges have and will continue to arise in judicial proceedings, in patent validity or infringement or challenge, inventorship priority contests and product liability actions. The key to winning in such cases is the ability to supply appropriate evidence.

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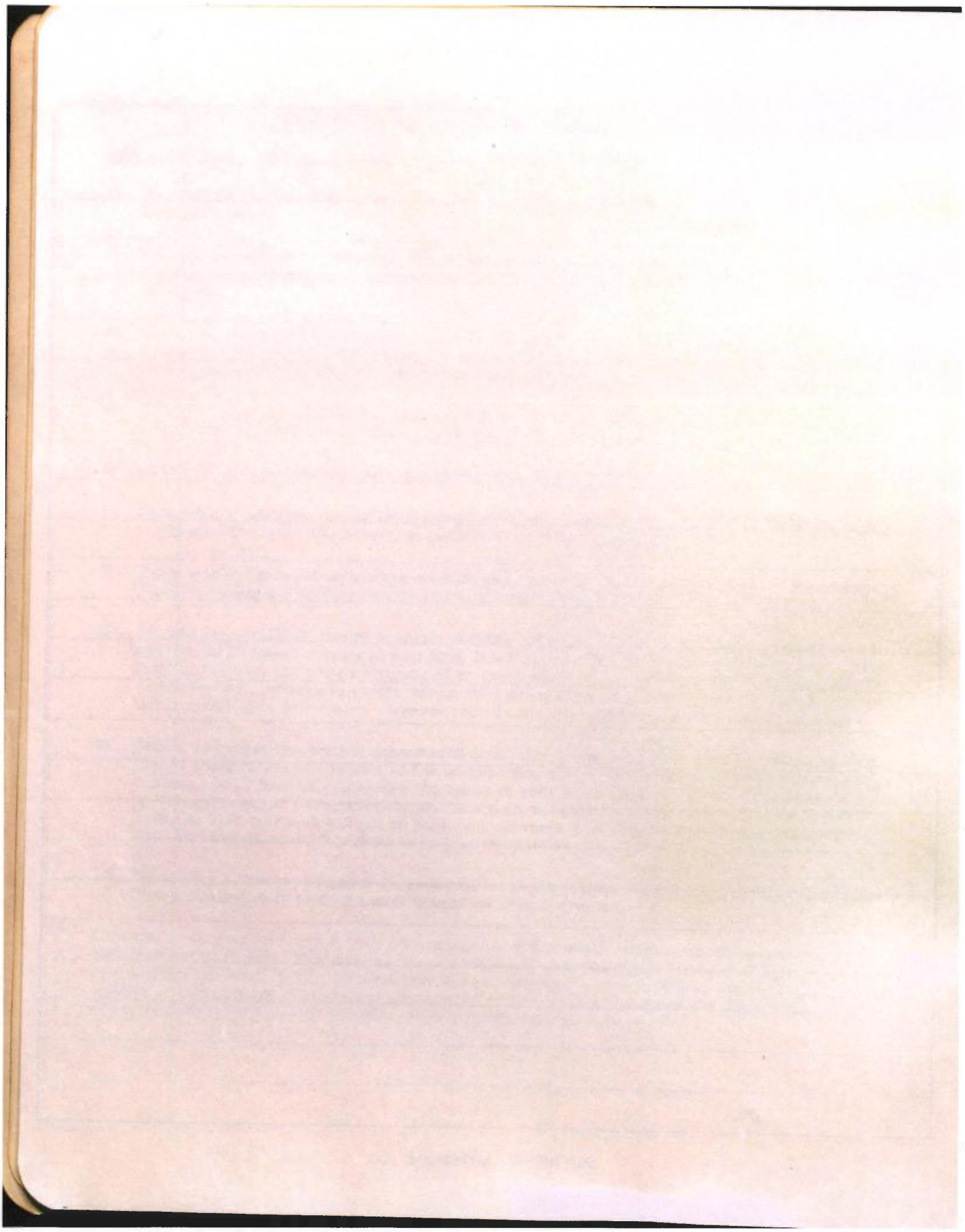

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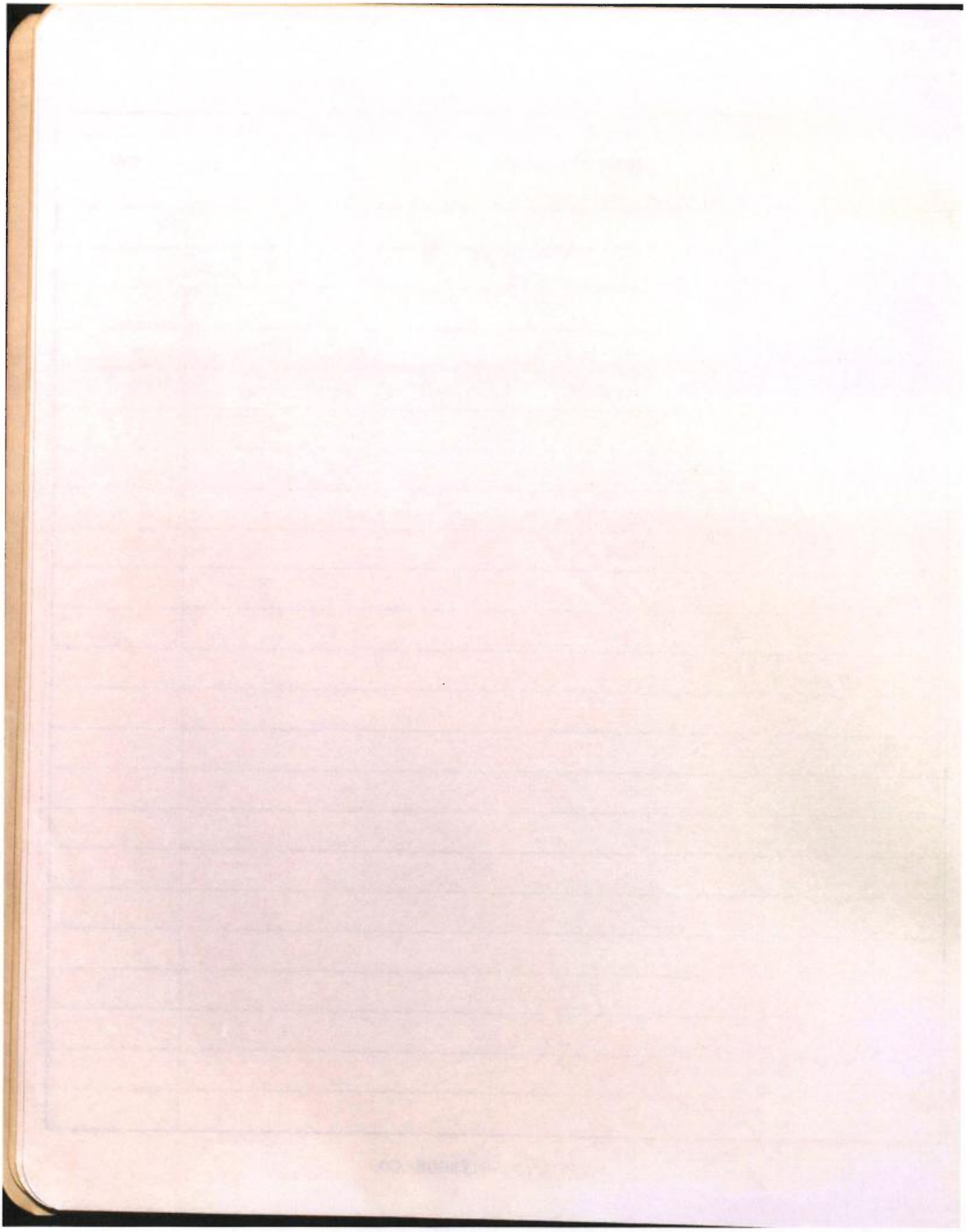

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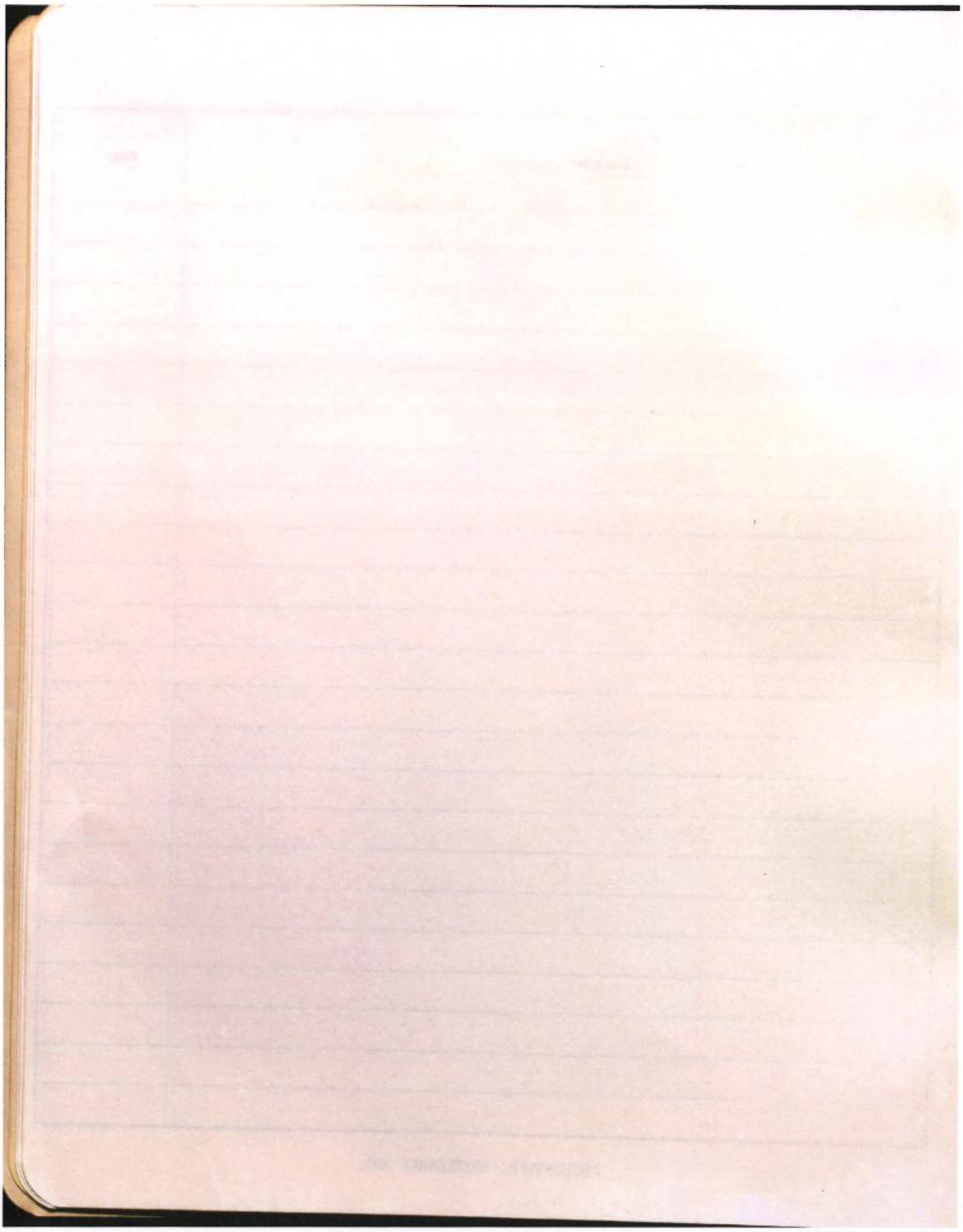

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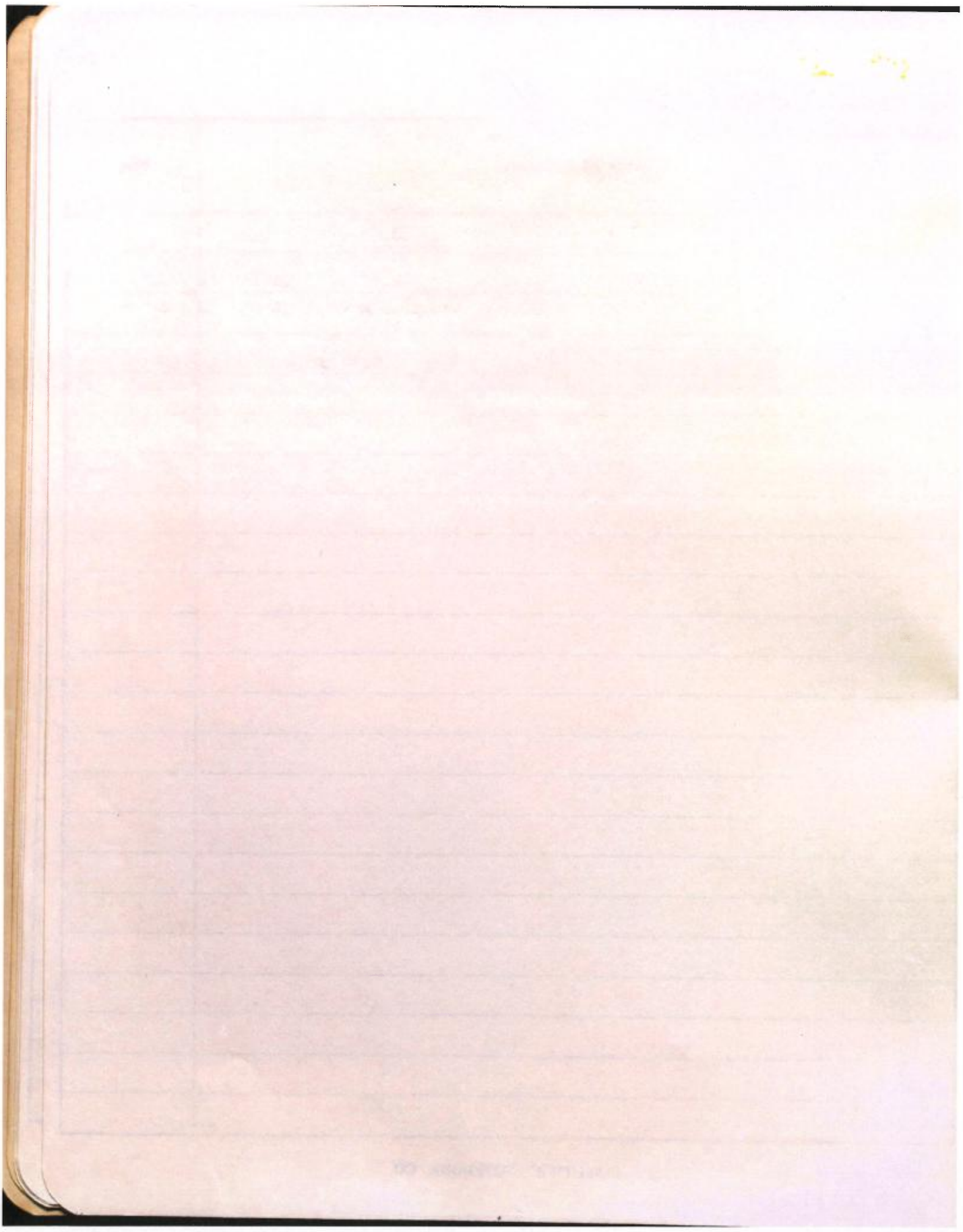

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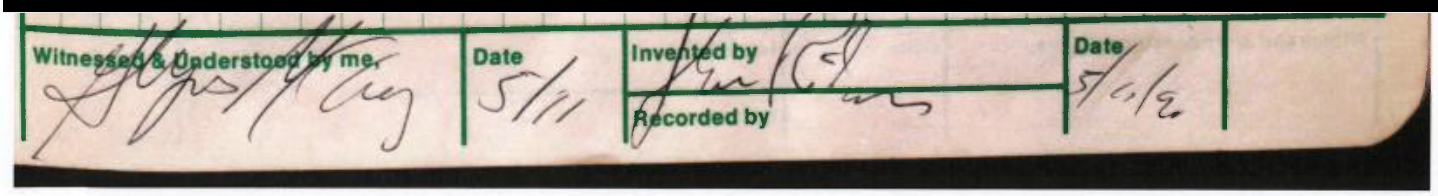
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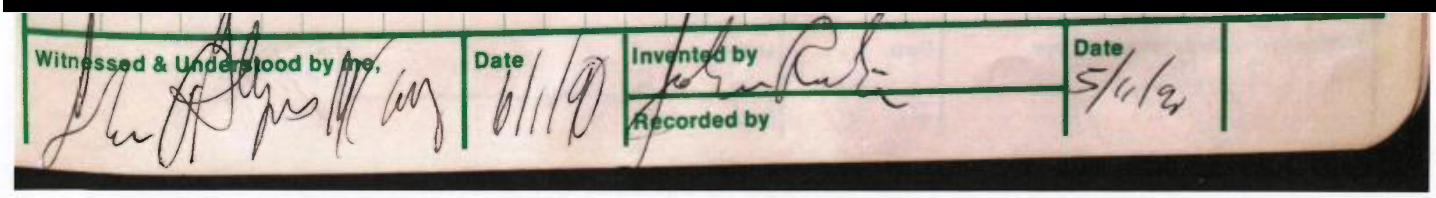
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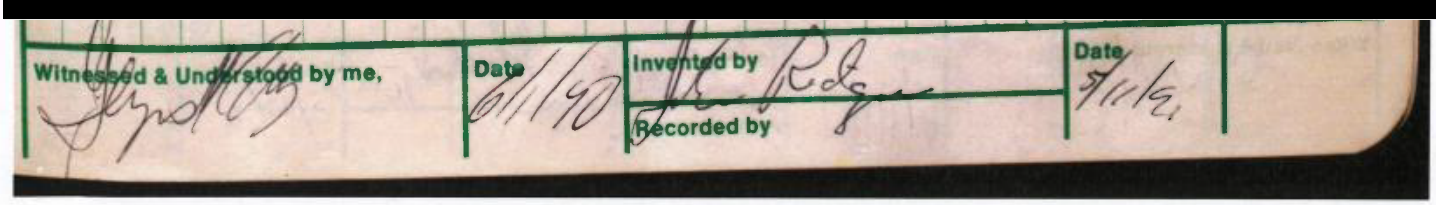
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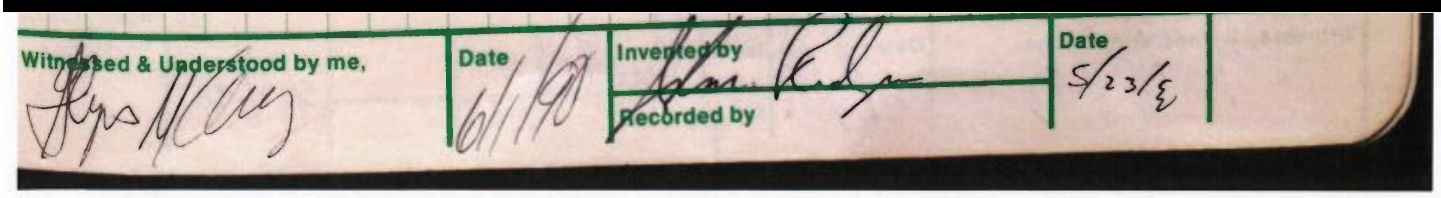
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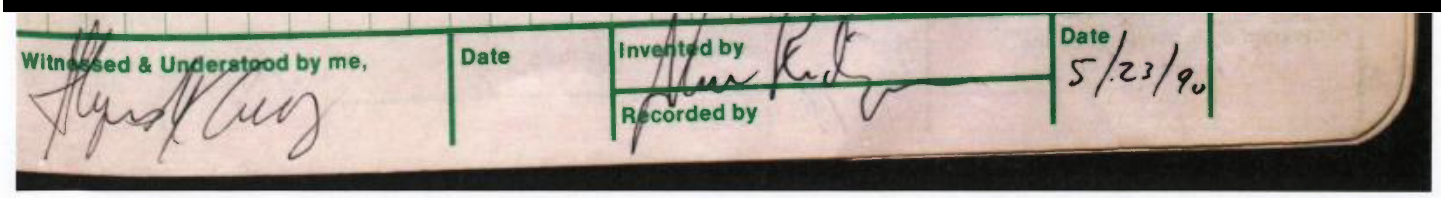
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	6/1/90		5/23/90
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Humanized 4D5 ("wild type") H+L

TLE 293 for transient Mutant Hu 4D5 Project No. Book No. 35

rom Page No.

5/9/90 1) pA5 Heavy I_gG1 Hu4D5 : 5'. pAK5. hu4d5hc
Clone 2/1 DNA prep 3.38 x/λ

2) pA3 Light Hu4D5-K1 : 5'. pAK5. hu4d5lc
clone 7 DNA prep 2.74 x/λ

4 x 100 mm² dishes : 10 mg/dish x 4 = 40 mg DNA total in 4 ml.

DNA ratio 4:4:2, pA5 : pA3 : AdVa 0.88 mg/ml.

16 mg pA5 ÷ 3.38 = 4.7 ml

16 mg pA3 ÷ 2.74 = 5.8 ml

8 mg AdVa ÷ .88 = 9 ml

DNA	1/10 TE	CaCl2	2x Hepes
pA5 - 4.7 ml pA3 - 5.8 ml AdVa - 9 ml	1.8 ml	200 ml	2.0 mls

Fast cells 10AM
pA on 1: PM
Shock 4: PM Refeed w/ 10% FBS-Hi

5/10/90 10AM change to Serum Free DMEM + Ins + Gln.
Plates very nearly confluent. 1 plate split 1→4. Seeded w/ Reg 10% FBS medium to cells settle. 4pm. Change 4 plate back to Serum Free + Ins + Gln
5/11/90 Fri Collected media Sup + re fed. all plates

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Witnessed & Understood by me, Date 6/1/90 Invented by Recorded by Date 5/23/90

Project No._____
Book No._____ TITLE 4D5 2.6 in MTX

From Page No.__

5/10/90 Count 56,860 x 40 = 2,274,400 /ml.

Seed at 5×10^7 in 0.25 ml/plate.

MTX at 2, 5, 10, 20 nM

Froze rest of cells 2 vials at 1×10^7 cells each

labled 4D5. 2.6 1M 5/14

5/14 fd.

To Page No.__

Witnessed & Understood by me, Date 6/1/90

Invented by John Ridgway Date 5/23/90

Recorded by

chimeric 405

TLE Thaw DP-12 405 (2.6) for MTX exp. Project No. Book No.

2.16 MTX Exp.

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om Page No.

5/9/90 Only 1 colony was produced by 10uM MTX so try again w/ less.

Thaw 1 vial from 4/17/90, into 3T 12.5. Cells look great > 90% viability.

Wait for recovery. Feed at 5×10^5 cells/well dish (Re-freeze 2 vials)

Use 2, 5, 10, 20 uM MTX + 0 uM TX Control

5/10/90 Trypsinize 1 plate resuspend in 10 ml.

Count 0.5 ml in 9.5 ml PBS = 20x dil machine count 0.5 ml

12,674 x 20 (dil) x 2 = 506,960 cells/ml

Use 1 ml/plate + 9 ml 4% FBS + Ch (no GHT)

Stock 1000uM MTX.

2uM: $\frac{2uM}{1000uM} \times 10{,}000ul$ = 20 ml of 1000uM MTX

5uM: $5/1000 \times 10{,}000$ = 50 ml of 1000uM MTX

10uM: use 100 ml 1000 uM MTX

20uM: use 200 ml 1000 uM MTX

50uM: use 500 ml 1000 uM MTX

there are 2.16 cells not 2.6

5/14 Fed

5/16 Fed

To Page No.

Witnessed & Understood by me. Date 6/1/90

Invented by

Recorded by

Date 5/23/90

Project No._____
Book No._____ TITLE ^{35}S labeling of 293 for 405 p35

From Page No.__

5/10/90 Transfection p35.

Label 1 100 mm^2 dish, 1 293 cell

2.5 ml label x 2 = 5 ml label

Use 100 uCi/ml cys, met ^{35}S.

= 500 uCi cys, met ^{35}S

Use Amersham label at conc of 1 mCi / 100 ul.

500 uCi / 10 mCi/ml = 50 ml 10 mCi / ml

Use 50 ml each of cys, met ^{35}S in 5 ml cys-, met- media.

See p. 51 for I.P. of Abmore.

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Witnessed & Understood by me, [signature] Date 6/1/90

Invented by [signature]

Recorded by

Date 5/23/90

From Page No. 35

5/14/90 Collected Sups from Fri 5/11 - Mon 5/14.
20 ml unsplit cells + 40ml 1→4 split cells.

Transient Humanized:
Assayed: IgG (Human) + Her-2 Binding (ELISA)

Unsplit 5/11	5.3 ug/ml	7.45 ug/ml	30 hrs
Unsplit 5/14	7.1 - 7.7 ug	12.5 ug	65 hrs
1→4 split 5/11	1 ug	1.44 ug	24 hrs
1→4 split 5/14	1.35 ug	2.24 ug	65 hrs

Control: 2.16 CHIMERA (Mouse Variable + Human Constant)
1.11 ug 1.92 ug

The Humanized looks very similar to the Chimera and the transfection was very good.

Other: IgG | Her-2 Binding

ASSAY RESULTS ARE NOT CORRECTED FOR DILUTION

NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION	ug/ml	NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION	ug/ml
1	1 unsplit 5/11/90	1/10	110	21			2.01
2	"	1/100	50.9	22			2.28
3	"	1/500	5.3	23			7.45
4	2 unsplit 5/14/90	1/10	>110	24			2.21
5	"	1/100	71.1	25			2.26
6	"	1/1000	7.7	26			12.5
7	3 1→4 5/11/90	1/10	112.6	27			2.28
8	"	1/100	11.5	28			2.26
9	"	1/1000	1.5	29			1.44
10	4 1→4 5/14/90	1/2	165	30			2.26
11	"	1/10	[illegible]	31			2.21
12	"	1/100	[illegible]	32			2.24
13	5 1→4 2.16	1/2	[illegible]	33			2.28
14	" 5/14/90	1/10	[illegible]	34	Shankya		2.24
15	"	1/100	[illegible]	35			1.92
16				36			
17				37			
18				38			

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Witnessed & Understood by me, [signature] Date 6/1/90 Invented by [signature] Recorded by Date 5/23/90.

Project No._____
Book No._____ TITLE Froze 405.2.16 Cells

From Page No.__

5/15/90 405 Chimera 2.16 : Froze 3 vials from 2 100 dish
Labled "405→2.16 chimera"

Results of 405-2.16 Elisa's
and 293 tfm of 405 for mike Shepard

Genentech, Inc.
ASSAY: Hu IgG Elisa

SAMPLES SUBMITTED BY	PROJECT NO.	PRODUCT
John R. Lowe		
EXTENSION: 1107	DATE SUBMITTED: 5/10/90	DATE TO BE ASSAYED: 5/10/90

☐ GMP ☐ BIOHAZARD
☐ GLP ☐ TOXIC HAZARD
☐ RADIO ACTIVE ☑ NONE OF THE ABOVE
☐ MAIL RESULTS* ☐ CALL WHEN READY
* RESULTS NOT PICKED UP BY END OF DAY WILL BE MAILED.

Sample Matrix: Hu IgG
Anticoagulant (if used): Elisa
Storage Conditions: ☐ Ambient ☐ 2°-8° ☐ -10° or Below
☐ Other:
NOTES:

NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION
1	405 2.16 5/14/90	1/10
2	"	1/50
3	"	1/100
4	"	1/1000
5	"	1/10,000
6	293 405 5/14/90	1/10
7	"	1/50
8	"	1/100
9	"	1/1000
10	"	1/10,000
11	293 405 5/7/90	1/10
12	"	1/50
13	"	1/100
14	"	1/1000
15	"	1/10,000
16		

Genentech, Inc.
ASSAY: Her-2 Binding

SAMPLES SUBMITTED BY	PROJECT NO.	PRODUCT
John R. Lowe		
EXTENSION: 1107	DATE SUBMITTED: 5/10/90	DATE TO BE ASSAYED: 5/10

☐ GMP ☐ BIOHAZARD
☐ GLP ☐ TOXIC HAZARD
☐ RADIO ACTIVE ☑ NONE OF THE ABOVE
☐ MAIL RESULTS* ☐ CALL WHEN READY
* RESULTS NOT PICKED UP BY END OF DAY WILL BE MAILED.

Sample Matrix:
Anticoagulant (if used):
Storage Conditions: ☐ Ambient ☐ 2°-8° ☐ -10° or Below
☐ Other:

NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION
1	405 2.16	1/10
2	"	1/50
3	"	1/100
4	"	1/1000
5	"	1/10,000
6	293 405 5/14/90	1/10
7	"	1/50
8	"	1/100
9	"	1/1000
10	"	1/10,000
11	293 405 5/7/90	1/10
12	"	1/50
13	"	1/100
14	"	1/1000
15	"	1/10,000
16		

Her-2
ECD binding.

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Witnessed & Understood by me, [signature] Date 6/1/90

Invented by [signature]
Recorded by

Date 5/23/90

TITLE Bio Assay w/ SKBR3 cells. Project No. Book No. 41

From Page No.

5/10/90 Submitted Sups of 4D5 Humanized + Chimeric Ab to the bio assay.

Dilutions made to 0.32, 0.16, 0.08, 0.04 ug/ml of ECD binding Ab, based on Swail Less Her-2 binding ELISA

4 Samples submitted:

1) 4D5 chimera: Mouse Variable region w/ Hu Constant region clone 2.16. Starting conc 4.24 ug/ml

First dilution to 0.32 ug/ml made as follows

4.24(x) = .32(5 ml) x = .377 ml

377 ml of 2.16 clone + 4623 ml diluent = 5 ml at 0.32 ug/ml

2) 4D5 chimera from 293 transient expression at 2.5 ug/ml

2.5x = .32(5) x = 0.640 ml QS → 5 ml

3) 4D5 humanized: mouse CDR w/ Hu Variable region backbone and Hu Constant region

293 trans exp 5.3 ug/ml IgG, 7.45 ug/ml ECD binding

7.45(x) = .32(10) x = .430 ml QS → 10 ml

4) 4D5 humanized: 293 transient expression. 2.24 ug/ml ECD

2.24(x) = .32(5) x = 0.714 ml QS → 5 ml diluent.

For all samples → 4, use 1:2 serial dilutions to 0.04 ug/ml.

Results p42, 43

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Witnessed & Understood by me, Glynis McCoy Date 6/1/90

Invented by Recorded by Date 5/16/90

42 Genentech, Inc.

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ASSAY REQUEST/REPORT FORM

GRAY TO BE COMPLETED BY ASSAY SERVICES	
A.S. EXPT NO. 4006-88	DATE
A.S. TECH. M.C	TEST PROCEDURE

ASSAY: Anti Her-2 Bioassay

SAMPLES SUBMITTED BY	PROJECT NO.	PRODUCT I.D.	COST CENTER NO.	TIME OF REPORT
John Ridgway			435	DATE
EXTENSION X1107	DATE SUBMITTED 5/16/90	DATE TO BE ASSAYED 5/17/90	NUMBER SUBMITTED	TIME / INIT.

CHECK ONE OF THE FOLLOWING:

- ☐ GMP ☐ BIOHAZARD
- ☐ GLP ☐ TOXIC HAZARD
- ☐ RADIO ACTIVE ☑ NONE OF THE ABOVE
- ☐ MAIL RESULTS* ☐ CALL WHEN READY

* RESULTS NOT PICKED UP BY END OF DAY WILL BE MAILED.

ASSAY STATUS AND SPECIFICATIONS

- ☐ Nonqualified
- ☐ Qualified/ Nonvalidated
- ☐ Validated

Control Value Expected A ____ B ____ C ____ D ____

Obtained A ____ B ____ C ____ D ____

Acceptable Range ____

Sample Matrix:

Anticoagulant (If used):

Storage Conditions: ☐ Ambient ☐ 2°-8°c ☐ -10°c or Below

☐ Other:

NOTES: Dilutions in ng/ml of ECD binding activity, from Wai Lee's Her-2 binding elisa.

ASSAY RESULTS ARE NOT CORRECTED FOR DILUTION

NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION	4D5 Conc (ng/ml)	% Inhib. CTR	NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION
1	4D5 Chimera	0.32	.30	53	21		
2	Clone 2.16	0.16	.16	74	22		
3	↓	0.08	.08	88	23		
4	↓	0.04	<.025	-	24		
5	4D5 Chimera	0.32	.10	84	25		
6	transient 293	0.16	<.025	-	26		
7	↓	0.08	<.025	-	27		
8	↓	0.04	<.025	-	28		
9	4D5 humanized	0.32	<.025	-	29		
10	transient 293 #1	0.16	<.025	-	30		
11	↓	0.08	<.025	-	31		
12	↓	0.04	<.025	-	32		
13	4D5 humanized	0.32	<.025	-	33		
14	transient 293 #4	0.16	<.025	-	34		
15	↓	0.08	<.025	-	35		
16	↓	0.04	<.025	-	36		
17	293 media CTR	1/5	<.025	-	37		
18	BP-12 media CTR	1/5	<.025	-	38		
19					39		
20					40		

5/23/90

73 (12/88)

TITLE 4D5 hybridoma standard for Bio Assay Project No._____ Book No._____ 43

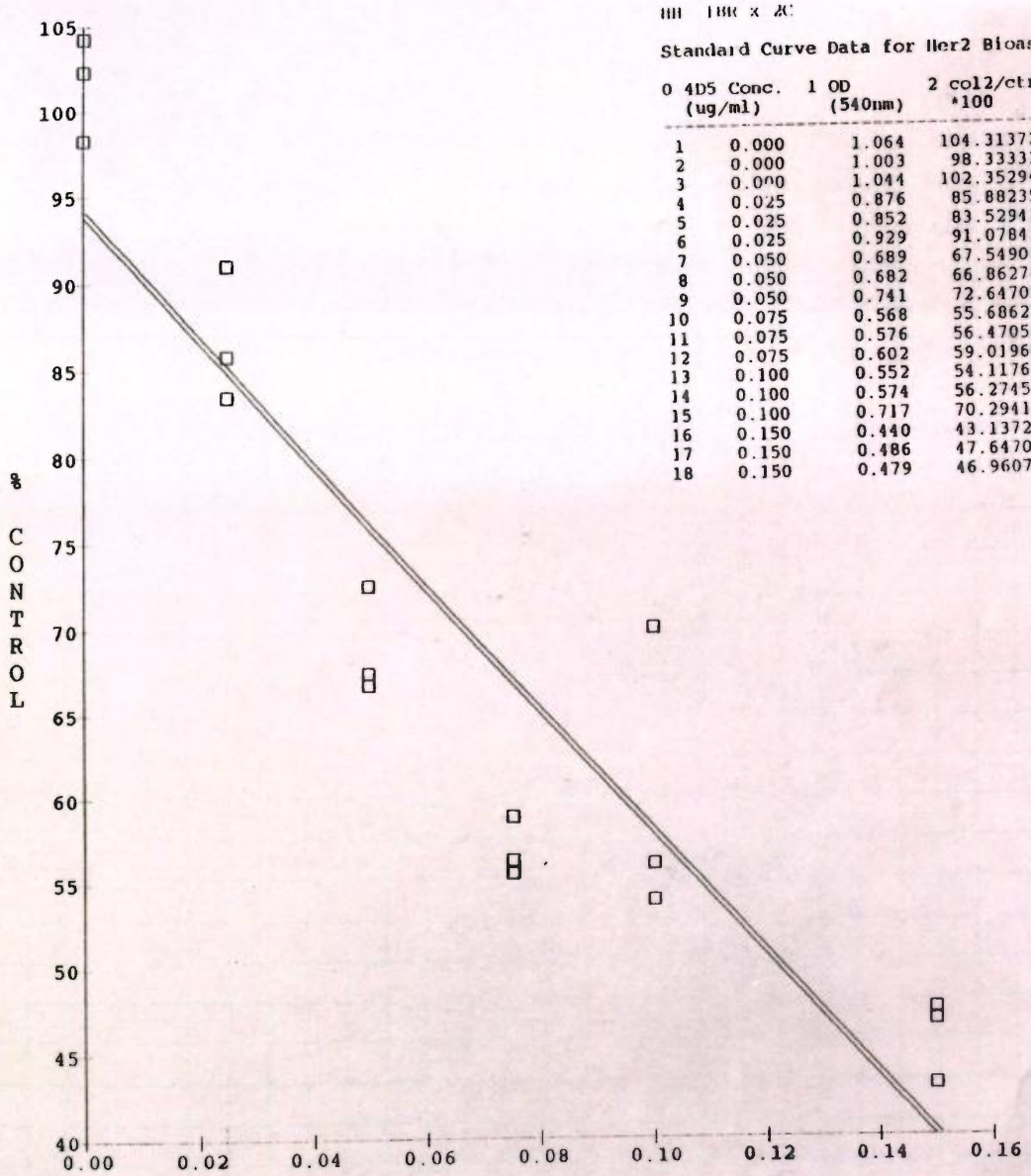
HHG 21-MAY-90 16:53 Page 1

4D5 Standard Curve

HH HBC x AC

Standard Curve Data for Her2 Bioassay 11006-88

0	4D5 Conc. (ug/ml)	1 OD (540nm)	2 col2/ctrl *100
1	0.000	1.064	104.313725
2	0.000	1.003	98.333333
3	0.000	1.044	102.352941
4	0.025	0.876	85.882353
5	0.025	0.852	83.529412
6	0.025	0.929	91.078431
7	0.050	0.689	67.549020
8	0.050	0.682	66.862745
9	0.050	0.741	72.647059
10	0.075	0.568	55.686275
11	0.075	0.576	56.470588
12	0.075	0.602	59.019608
13	0.100	0.552	54.117647
14	0.100	0.574	56.274510
15	0.100	0.717	70.294118
16	0.150	0.440	43.137255
17	0.150	0.486	47.647059
18	0.150	0.479	46.960784



To Page No.___

Witnessed & Understood by me,	Date	Invented by	Date
[illegible]	6/1/90	Recorded by:	5/23/90

From Page No.

5/23/90 Bioassay Done 5/17 - 5/22 to be repeated w/ Samples Concentrations based on IgG elisa data not ECIS binding

Sample 1: 405 chimera Stable line 2.16. Sup from 5/6/90 (p.40) By IgG Elisa: 4.5ug/ml.

Dilute to 1.28ug/ml 4.5(x) = 1.28(5ml)
x = 1.42
1.42ml Sup + 3.58ml diluent
= 5mls at 1.28ug/ml

Dilute 1:2 Fin Conc. .64, .32, .16, .08, .04ug/ml

Sample 2: 405 chimera From 293 trans 3/9/90 2.5ug/ml IgG
make dilutions 1.25 → .04ug/ml

Sample 3: Humanized 405 - Version(a) From 293 trans 5/14/90 Sup 39.
By IgG Elisa: 7.1ug/ml

Dilute to 2.56ug/ml 7.1(x) = 2.56(5ml)
x = 1.8ml + 3.2ml diluent
= 2.56ug/ml

Make 2.56 → .04ug/ml dil.

To Page No.

Witnessed & Understood by me, Date 6/1/90 Invented by Recorded by Date 6/1/90

Project No._____

TITLE_____ Book No._____ 45

From Page No.___

To Page No.____

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	

46

Project No._____

Book No._____ TITLE _____

From Page No.__

To Page No.__

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	

From Page No.

5/23/90 All Samples in 1/10, 1/100, 1/1000 dil

Samples: 100ng/ml 1) 4D5 2.6 10nM MTX - Started 5/10/90

2) 4D5 2.6 20nM MTX - "

3.3ug/ml 3) 4D5 2.6 10nM MTX clone selected From Amp Started 4/18

3.4ug/ml 4) Same as #3 - 3-4day culture.

1.9ug/ml 5) 4D5 2.16 5nM MTX - started 5/10/90. - this culture looks partially selected - too many clones

600ng/ml 6) 4D5 2.16 10nM MTX - 5/10 - in complete killing

200ng/ml 7) 4D5 2.16 20nM MTX - 5/10

4ug/ml 8) 4D5 2.16 No MTX >5day Amps.

3.6ug/ml 9) 4D5 2.16 5/6/90 used in Bioassay

1.7ug/ml 10) 4D5 293 trans 5/9/90 used in Bio Assay

7.3ug/ml 11) Humanized 4D5 pA3, pA5 293 trans From 5/14 #2 used in BioAssay

290ng/ml 12) 4D5 2.16 O.N. on cells in 100mm dish to test if cells are still producing Ab. (weak cells)

10nM MTX Amp: 520ng → 13) 4D5-2.6 - 10^5 start seed Bx Cell Assay 3days all cultures.

1.3ug → 14) 4D5-2.6 - 10^6 "

2ug → 15) 4D5-2.16 - 10^5 "

4.5ug 16) 4D5-2.16 - 10^6 "

Results next page →

To Page No.

Witnessed & Understood by me, [signature] Date 6/15/90

Invented by [signature] Date 6/15/90

Recorded by [signature]

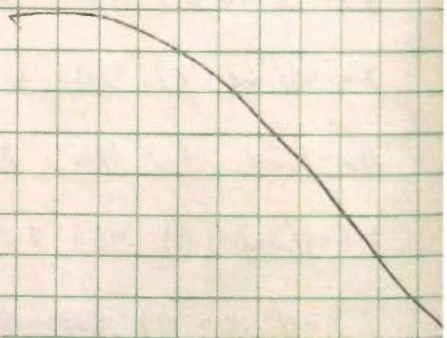
Project No._____
Book No._____ TITLE Igb Elisa Results. from p47

From Page No. 47

Results.

1.) 100 ng/ml
2.) -0-
3.) 3.3 ug/ml
4.) 3.4 ug/ml
5.) 1.9 ug/ml
6.) 600 ng/ml
7.) 200 ng/ml
8.) 4 ug/ml
9.) 3.6 ug/ml
10.) 1.7 ug/ml
11.) 7.3 ug/ml
12.) 290 ng/ml
13.) 570 ng/ml
14.) 1.3 ug/ml
15.) 2 ug/ml
16.) 4.5 ug/ml

Per Cell Assay a b c d



To Page No._____

Witnessed & Understood by me, [signature] Date 6/15/90

Invented by [signature]
Recorded by

Date 6/15/90

From Page No.

5/26/90 ~Noon

Cell Counts: 1/200 dil x 0.5 ml count = 2951

2.16 → 2951 x 400 = 1.18×10^6 cells/ml

$5 \times 10^5 / 1.18 \times 10^6$ = 0.42 ml/dish

Set up 2 dishes 1) 5×10^5, 2) 1×10^6

2.6 (10 MTX): 1/200 dil, 1/2 ml count: 3037

3037 x 400 = 1.21×10^6 = 0.41 ml/dish

Set up 2 dishes 1) 5×10^5, 2) 1×10^6

5/29/90 Cell Counts:

a) 2.6 (10 MTX) - 5×10^5 seed - 1/100 dil = 1034 x 10 mls.
1034 x (2 x 100/2000 x 10 mls) = 2.068×10^6 cells

b) 2.6 (10 MTX) - 1×10^6 seed - 1/100 dil = 2500 x 10 mls.
2500 x 2000 = 5×10^6 cells

c) 2.16 - 5×10^5 seed - 1/100 dil = 1288 x 10 mls.
1288 x 2000 = 2.576×10^6 cells.

d) 2.16 - 1×10^6 seed - 1/100 dil = 2575 x 10 mls.
2575 x 2000 = 5.15×10^6 cells.

Results

a) 2.6 (10 MTX): 0.09 Picograms · cell^{-1} day^{-1}
b) 2.6 (10 MTX): 0.38 " "
c) 2.16 : 0.36 " "
d) 2.16 = 0.29 " "

Forgot 10x factor (10 ml of Sup)

To Page No.

Witnessed & understood by me: Date 6/15/90

Invented by Recorded by Date 6/15/90

Project No._____
Book No._____ TITLE Per Cell Assay Results

From Page No.__

2.6 10nM MTX
2.16 No MTX.

a) 2.6 10nM MTX 5×10^5 Seed : 570 ng/ml x 10 ml = 5.7mg Ig6

5.7ug = 5,700,000 ng / 2.068×10^6 cells · 3 days = 0.9 pg/cell·day

b) 2.6 10nM MTX 1×10^6 Seed: = 0.8 pg/cell·day.

c) 2.16 5×10^5 Seed = 2.6 pg/cell·day

d) 2.16 1×10^6 Seed = 2.9 pg/cell·day

To Page No.__

Witnessed & Understood by me,	Date	Invented by	Date
	6/15/90	Recorded by	6/15/90

From Page No. 38

5/30/90 Scored exp of cells + Sups

a) 293 CTA cells
b) " " Sups
c) 293 4D5 pA3,5 cells
d) " " Sups

Incubated w/ 3ml of Rd Human Hi Ig 30 min → 1 hr
Stopped cells - 50ml ↓ 30 min
Washed as per I.P. protocol

Resuspended in 80ml Sample buffer

Run 10ml/lane Reduced + Non Reduced See NB 10840 p72

To Page No.____

Witnessed & Understood by me, [signature] Date 6/15/98

Invented by [signature]

Recorded by

Date 6/5/90

52 Project No.____ Book No.____ TITLE Bio Assay Spike Exp w/ 293 Sups

From Page No.__

6/1/93 Concern that dde Sups from 293 transfections of Chimera 4D5Hu are degrading the Ab, resulted in this exp

ASSAY RESULTS ARE NOT CORRECTED FOR DILUTION

NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION	ug/ml	% CTRL	NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION	ug/ml	% CTRL
1	4D5 2.16 Stk 6	1:28	>.204	44.3	21	Chimera 4D5	5.5	>.204	41
2		0.64	.379	51.2	22	"	0.55	.329	57
3		0.32	.367	65.4	23	"	0.25	.266	66
4		0.16	.112	64.9	24	# 22 in #5 batch	0.55	.194	79
5	293 chimera 1:2		<.025	94.9	25	# 23 "	0.25	.146	81
6	#5 w/0.64 & 2.16		.198	74.0	26				
7	Hu #1 (293)	1:2	<.025	109	27				
8	" #2	1:2	<.025	162	28				
9	" #3	1:2	<.025	95	29				
10	#4	1:2	<.035	154	30				
11	293 chimera (panel)	1/10	.249	55	31				
12		1/20	.345	56	32				
13		1/40	.356	54	33				
14		1/80	.345	56	34				
15		1/160	.357	67	35				
16	#11 in #5 batch	1/10	.071	90	36				
17	#12 "	1/20	<.035	98	37				
18	#13 "	1/40	<.035	99	38				
19	#14 "	1/80	<.035	84	39				
20	#15 "	1/160	<.035	104	40				

73 (12/88)

RETURN TO REQUESTOR

Samples 1-4 Good CTO 2.16 material.
5 - Bad Sups - No activity.
6 - 2.16 Spike is inhibited by 293 Sups
7-10 Humanized transients - No activity perhaps due to degradation
11-15 Ab made in 293 cells - Pur. Final - works fine.
16-20 293 Sups totally inhibits Ab.

To Page No.__

Witnessed & Understood by me, | Date | Invented by John Rudgers | Date 8/10/93

Recorded by

From Page No.

6/1/90 1 confluent 175 cm² flask to 2 Roller bottles w/ 2 used media. ~1:60 split + 10mM Hepes

6/4/90 Cells good- ~70-80% confluent. Change to Serum Free DMEM + Insulin + Glu 250 ml/bottle w/ 10mM Hepes

6/7/90 Harvested both bottles. Submitted for IgG Assay. Refed w/ same media as above.

Results IgG - 1 mg/l - 0 mg/ml

6/13/90 Many more cells in Sup. Removed the media - Spun down cells (0.1 pellet) Rinsed media to clean containers

Saved Used Bottle 6/7 and 6/13 harvest to Reg Blank for purification on Step A Column.

6/7 Purified : 113 ug/ml

NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION	ASSAY RESULTS ARE NOT (4D5 Conc ug/ml)
1	2.16 Harvest 6/7/90	1/5	.215
2	Transient 6/8/90	1/5	~~1.085~~
3	Purified 6/7/90	1/5	2.025
4			
5			

R&D Assay results of 2.16 roller bottle harvest. 1/5 dil is ~0.2 ug/ml ∴ it works as well as 4D5 at this low concentration.

~~IgG~~ IgG Elisa results for R.B 6/13/90 harvest - 240 ng/ml

To Page No.

Witnessed & Understood by me, Date 6/15/90

Invented by Date 8/1/90

Recorded by

Project No._____
Book No._____ TITLE 293 transient chimeric 4D5H+L

From Page No.__

Fed Cells 9:15 ~70% Confluent.

6/5/90 Make more transient chimeras and purify on Prot A right after harvest F.

4 100 dishes using 10 ug/dish in 1 ml.

40ug DNA in 4 mls.

L chain pdH158 =	0.4ug/ml	(4.5)(4) ÷ 0.4 =	45ml
H chain pdH160 =	1.3ug/ml	(4.5)(4) ÷ 1.3 =	13.8ml
helper AdVA =	0.25ug/ml	1 (4) ÷ 0.25 =	16ml
			75ml DNA

Ratio of 4.5 : 4.5 : 1
(158) : (160) : AdVA

DNA	1/10TE	CaCl2	2 x Hepes
75ml total.	1.725ml	200ml	2.0ml

Pgt on 12:15
Stocked 5:15

6/6/90 AM took Sup sample and changed media to S.F.

6/7/90 Collected S.F. media from 2 of 4 plates labeled: chimeric 293 24hrs.

6/8/90 Collected S.F. from remaining 2 plates labeled Chimeric 293 48 hrs.

Stained plate w/ RαHIgGH+L. Result: + ? CTR 293 - —

Slot Assayed for IgG - Result : Zero

To Page No.__

Witnessed & Understood by me,	Date	Invented by	Date
	6/15/90	Recorded by	8/10/90

|| 293 Transient 4D5 transfections

TITLE ~~Chimeric~~ + Humanized

Project No.______

Book No.______

From Page No.__

6/14/90

plasmids:
1) pA3 - Hu 4D5-K₁ (Light) - "La" 2.74 ug/λ
2) pA5 - Hu 4D5-H (Heavy) - "Ha" 3.38 ug/λ

3) pA3 - Hu4D5K₁ - Light - Lb - 1.3 ug/λ (3-b-14)
4) pA3 - Hu 4D5K₁ - Light - Lc - 4.7 ug/λ (3-c-[redacted])

5) pA5 - Hu 4D5-H - Heavy - Hb - 2.0 ug/λ (5-b-4)
6) pA5 - Hu 4D5-H - Heavy - Hc - 3 ug/λ (5-c-4)

7) pdH158 - L chain chimera - 0.4 ug/λ
8) pdH160 - H chain chimera - 1.3 ug/λ
9) AdVa 0.25 ug/ml

DNA	1/10 TE	CaCl₂	2X Hepes
Xfn 1. Humanized La + Ha 1 ul - La 1 ul - Ha 4 ul AdVa	225 ul	25 ul	250 ul
Xfn 2: La + Hb 1 ul - La 1 ul - Hb 4 ul AdVa			
Xfn 3: La - 1 ul Hc - 1 ul AdVa - 4 ul			
Xfn 4 Lb - 3 ul Ha - 1 ul AdVa - 4 ul.			

5 ug DNA total
in 0.5 ml total

Put on 12:45
Shock at 4:30

Re feed 10% FBS
w/media

Xfn 5 Lc - 1 ul
Ha - 1 ul
AdVa - 4

Xfn 6 Lc - 1 ul
Hc - 1 ul.
AdVa - 4

Xfn 7 Lb - 3 ul
Xfn 8 Lc - 1 ul

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Witnessed & Understood by me. [signature]

Date 6/15/90

Invented by [signature]

Recorded by

Date 8/10/9.

Project No._____
Book No._____ TITLE 293 Trans Xfxn Humanized

From Page No.__

6/15 Am. Many cells floating in most dishes
- take Samples of Sups from 1-6 and give to IgG Elisa and BioAssay Refed w/ S.F. media + Insulin
- Stain #7, 8 for L chain

Staining Results #7 Xfxn: L chain Version b - prep 3-b-14
Staining was extremely low - next to nothing

#8 L chain Version c - prep 3-c-7
Stained Very Well.

Note: DNA for #7 (3-b-14) had very little Supercoiled compared to #8 (3-c-7)

See DNA Gel for these and others run on Cesti NB10840 p77

6/17/90 Removed S.F. media (Kept) Spun out cells. Submit to IgG Elisa on Monday (6/18)

6/18/90 Results: # by Xfxn Sup55.

ASSAY RESULTS

NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION	ng/ml
1	#1	1/10	133.2
2		1/100	14.6
3		1/1000	1.4
4	#2	1/10	>180
5		1/100	36.9
6		1/1000	3.9
7	#3	1/10	>180
8		1/100	59.2
9		1/1000	5.6
10	#4	1/10	102.0
11		1/100	11.2
12		1/1000	1.0
13	#5	1/10	>180
14		1/100	29.8
15		1/1000	3.1
16	#6	1/10	>180
17		1/100	80.3
18		1/1000	8.3
19			
20			

RETUR

48hr Sups

To Page No.____

Witnessed & Understood by me, Glyn McCoy Date 6/15/90

Invented by [illegible] Date 8/1/90
Recorded by

TITLE 293 Hu 4A5 Variant Xfns

Project No.

Book No.

From Page No.

6/19/90 Fed Cells 10AM

Repeat from p 55

2 100 dishes each. = 2ml pel w/ 20ug DNA

1) Repeat Xfn #1

#1 DNA	½ TE	$CaCl_2$	2× Hepes
pA3-La - 4ml pA5-Ha - ~~3ml~~ AdVA - 4ml	~~0.9 ml~~	100ml	1ml.
2) Repeat Xfn #2 pA3-La - 4ml Hb - ~~3ml~~ 5ml AdVA - 4ml	0.9	100ml	1ml
3) Repeat Xfn #3 pA3-La - 4ml Hc - ~~4ml~~ 4ml AdVa - 2ml	0.9 ml	100ml	1ml

Return 2:15

Shocked at 6AM (pH looks o.k. Light but fine)

6/20/90 Changed to S.F. media w/ 2% Insulin. AM

6/21/90 AM Harvested Sup from 6 plates - Refed w/ S.F. 1 plate, stained others.

See pH A Purification

Stained w/ R+H(H+L) Ab 1:500 on 9AM

Xfn:

1) Slightly - but definitely some.
2) Staining well
3) Fair not as well as #2

To Page No.

Witnessed & Understood by me,

Date 9/7/90

Invented by

Recorded by

Date 9/10/90

Project No._____

58 Book No._____ TITLE Mycoplasma test. Results

From Page No._____

6/20/90 Submitted 405 clones 2.6 2.16 to Mycoplasma test on 6/4/90

ASSAY REQUEST/REPORT FORM

Genentech, Inc.

ASSAY: Mycoplasma

SAMPLES SUBMITTED BY: John Ridgway	EXTENSION 1107	MAILBIN # 40	COST CENTER 435
DATE SUBMITTED 6/4/90	DATE TO BE ASSAYED 6/5/90	☑ MAIL RESULTS* ☐ CALL WHEN READY ☑ ELEC. MAIL (Log in: JBGR) *Results not picked up by end of day will be mailed	
SAMPLE MATRIX CHO cell Sup w/ 1% FBS	ANTICOAGULANT (IF USED)	☐ GMP ☐ GLP ☐ RADIOA *Specify type and amount:	
SAMPLES OF HUMAN ORIGIN? ☑ NO ☐ YES IF YES, IDENTIFY SOURCE:		(SPECIFY TISSUE	
NOTES:			

ASSAY STATUS AND SPECIFICATIONS

☐ NON-QUALIFIED	CONTROL VALUE EXPECTED A_____ B_____ C_____
☐ QUALIFIED/ NON-QUALIFIED	CONTROL VALUE OBTAINED A_____ B_____ C_____
☐ VALIDATED	ACCEPTABLE RANGE:

NOTES:

ASSAY RESULTS ARE NOT CORRECTED FOR

NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION	RESULT		NO.	IDENT ORIGIN AND/
1	405 2.6	—	NEG		21	
2	405 2.16	—	NEG		22	
3					23	
4					24	
5					25	
6					26	
7					27	
8					28	

To Page No._____

Witnessed & Understood by me, [signature] Date 9/7/90

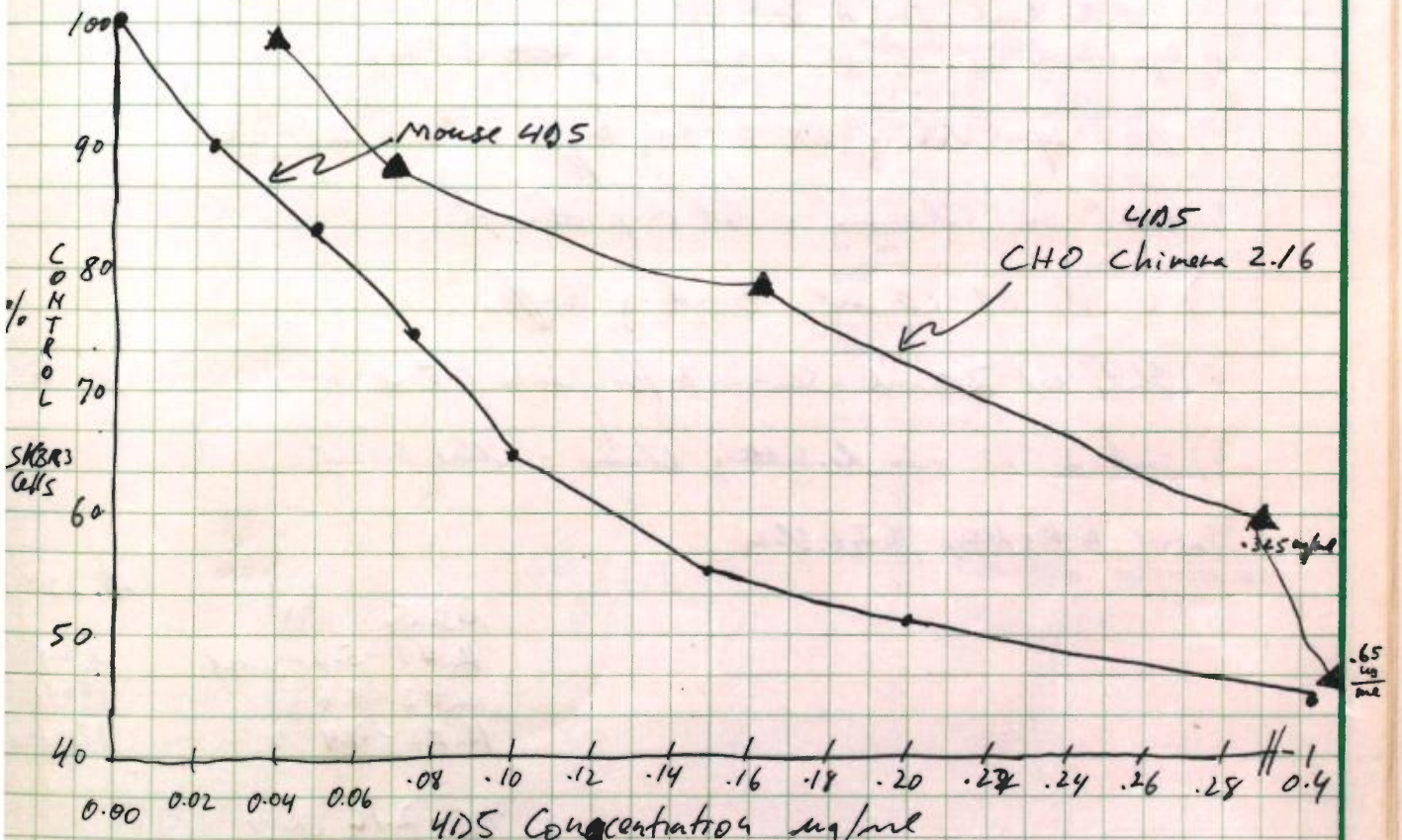
Invented by [signature] Date 8/6/90

Recorded by

TITLE Her-2 Bio Assay SKBR3 Cells Project No. Book No. 59

From Page No.

6/20/90 4D5 CHO 2.16 Stable Purified from Roller Bottle.



To Page No.

Witnessed & Understood by me, Date 9/7/90 Invented by Date 8/10/90 Recorded by

Project No._____
Book No._____ TITLE Prot A Purification of Hu 4D5 Abs

From Page No.__

6/21/90 Harvested Supe from 293 xfn 6/11 p57
3 xfn w/ 20 ml each in S.F. media

0.5-1 ml Prot A bed Vol

1) Equilibrate w/ 10 ml 1X binding ~~buffer~~ buffer.

2) Add equal Vol of 2X binding buffer to eq. Sample.

3) Load on Column - let clip through.

4) Wash w/ 10 ml binding buffer.

5) Elute w/ 20 ml Elution buffer into 0.5 ml 1M Tris-HCl ph 8.0

6) Load 2.5 ml onto de-Salting Column - Collect 3.5 ml.

Submit to Bio Assay, Hu IgG Elisa

NOTES: Hu #1, 2, 3 aa NB "Tosa Cells" 6/17/90 Transfer
6/24/90 Hu IgG Elisa

ASSAY RESULTS ARE NOT CORRECTED FOR DILUTION

NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS		DILUTION	ng/ml		NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION	ng/ml
1	Hu #1	Purified	1/10	>180		21	↓	1/400	20.6
2	293	6/21/90	1/100	49.5		22	↓	1/1000	2.0
3			1/1000	5.2		23	4D5 2.6-C	1/10	34.0
4		↓	1/10000	<.5		24	10 MTX	1/100	3.6
5	Hu #2	Purified	1/10	>180		25		1/1000	2.5
6	293	6/21/90	1/100	85.2		26			
7			1/1000	8.6		27			
8		↓	1/10000	<.5		28			
9	Hu #3	Purified	1/10	>180		29			
10	293	6/21/90	1/10²	[illegible]	43.2	30			
11			1/10³	[illegible]	4.4	31			
12		↓	1/10⁴	[illegible]	<.5	32			
13	4D5 2.16		1/10	[illegible]>180		33			
14	Roller Bottle		1/10²	>180		34			
15	Purified		1/10³	113.2		35			
16		↓	1/10⁴	11.9		36			
17	4D5 2.6-a		1/10	5.4		37			
18	10 MTX		1/100	[illegible].55		38			
19		↓	1/1000	<.5		39			
20	4D5 2.6-b		1/10	>180		40			
	10 MTX								

SERVICES

results: IgG Elisa ~~Hu~~ 280

Hu #1	5.2 ng/ml	23 ng/ml
Hu #2	8.6 "	18 ng/ml
Hu #3	4.4 "	18 ng/ml

4D5-2.16 clone Roller Bottle 3 day growth 113 ng/ml (Purified on Prot A Column) yield ~ 1 mg/liter.

To Page No.__

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	9/7/90	[signature]	8/10/90
		Recorded by	

TITLE [Protein IgG] determination w/ Spectrophotometer

Project No.

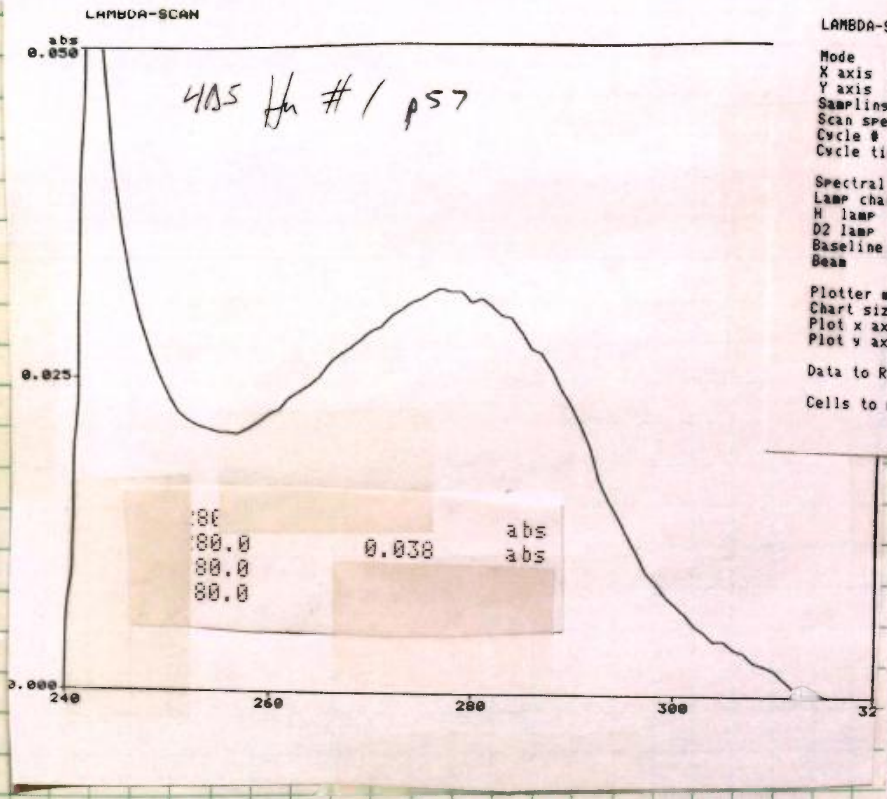
Book No.

61

From Page No.

6/20/90

Concentration after purification and Concentration 20 ml → 3.5 ml



LAMBDA-SCAN PARAMETER

Mode	abs
X axis min * max	240*320 nm
Y axis min * max	0.000*0.040 abs
Sampling interval	1.000 nm
Scan speed	120 nm/min
Cycle #	1
Cycle time	0.1 min
Spectral bandwidth	2.0 nm
Lamp change	340 nm
H lamp	on
D2 lamp	on
Baseline corr	off
Beam	double
Plotter mode	off
Chart size	20 cm
Plot x axis incr	off
Plot y axis incr	off
Data to RS 232C	off
Cells to measure	4

KONTRON UVIKON 860

Date: 21.06.90 Time: 17:08 Operator: ...

Sample Identification: .....

LAMBDA-FIX PARAMETER

Mode	abs
Wavelength	280.0 nm
Integration time	5.0 sec
Cycle #	1
Cycle time	0.1 min
Spectral bandwidth	2.0 nm
Lamp change	340 nm
H lamp	on
D2 lamp	on
Beam	double
Plotter mode	off
Data to RS 232C	off
Cells to measure	4

Formula of Conversion using Coef. and Extinction

For W.T. 405 Hybridoma #6.

1.65 OD_{280} = 1 mg/ml protein

Hu Ran #1

.038 Abs / 1.65 = .023 mg/ml

or

(2.3 mg/ml)

By IgG ELISA: (5.2 mg/ml)

To Page No.

Witnessed & Understood by me,

Date 9/7/90

Invented by

Recorded by

Date 8/10/90

Project No._____
Book No._____

TITLE MTX Amplification of 2.6

405

From Page No.___

6/22/90 Repeat of Amp by MTX at
10ng/ml MTX : yield 3 colonies

Ig G Elisa
a) 50ng/ml
b) 2ng/ml
c) 360ng/ml

See Copy of Assay p 60.

Results: No amplification.

To Page No.___

Witnessed & Understood by me,	Date	Invented by	Date
	9/7/90	Recorded by	8/10/90

TITLE Protein gel of Hu Var 1, 2, 3 4D5 Project No. Book No. 63

From Page No.

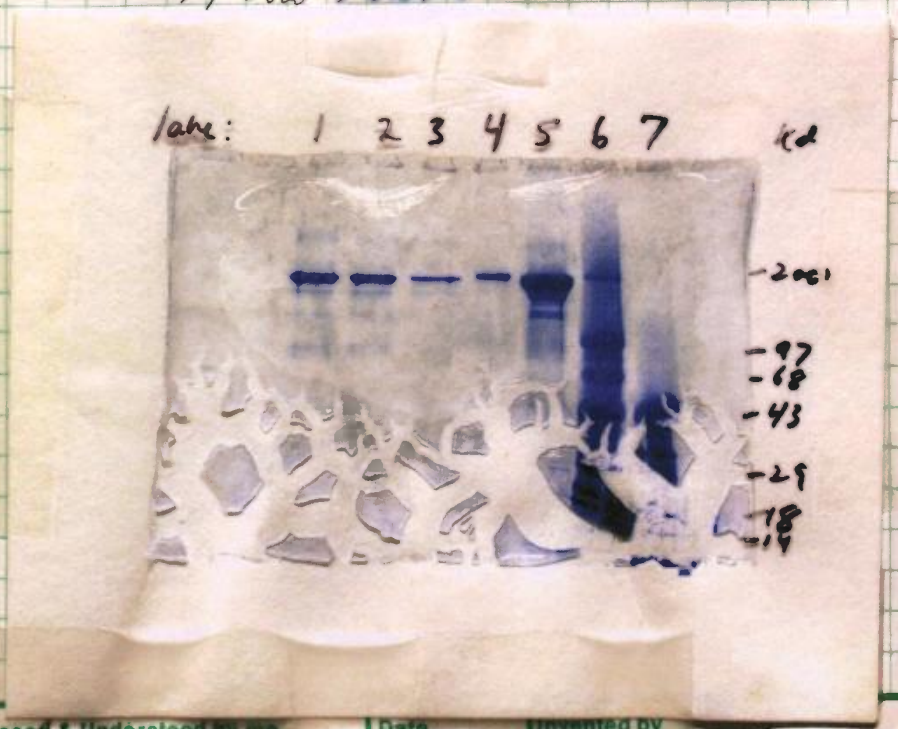
6/25/90 Precipitated 1 ml of Ab from P60.

Then were ~~pi~~ purified on ProA + Conc. to 7.5 Calculated Elisa P6!

(1 ml Ab + 4 ml Acetone. 15 min post time.
Spun in glass tube 10 min 10K rpm

Run on 4-20% Acrl gel pre cast
(Suspended in ~~to~~ ~~[illegible]~~ ja 10 ml SDS buffer + Coomassie 2x Gel buffer

Lanes) 1) Var 1 ≃ 5 ug (Run Non Reduced)
2) Var 2 ≃ 8.6 ug
3) Var 3 ≃ 4.4 ug
4) 4D5 Chimera - 10 ml of .113 mg/ml ≃ 1 mg
5) Mouse 4D5 - ~~[illegible]~~ ≃ 10 ug
6) High MWM 7 ul
7) Low MWM 7 ul



Results:
Ab protein of all 3 Var and Chimera is intact at time of Bio Assay

To Page No.

Witnessed & Understood by me. Date 9/7/90 Invented by Recorded by Date 8/10/90

Project No._____
Book No._____ TITLE Kinetics of ADS secretion Exp

From Page No.__

6/26/98 Transfect 8 T-60i w/ Hu Vax #1 (pA3, pA5)

$\frac{0.5\text{ml pet}}{60\text{ plate}} \times 8 = 4\text{ml pet}$

10mg DNA/ml × 4ml = 40mg DNA total + AdVA

20mg pA3 2.74mg/ml = 7.3ml
20mg pA5 3.38mg/ml = 6ml
4mg AdVA 0.88mg/ml = 5ml

DNA	1/10 TE	CaCl2	2X Hepes
	1.8mls	0.200ml	2mls
7.3ml pA3			
6ml pA5			
5ml AdVA.			

Hyb on 12:35
Shocked 4:30

To Page No.__

Witnessed & Understood by me, [signature] Date 9/7/98

Invented by [signature] Date 8/10/00

Recorded by

TITLE Pulse Chase for Secretion Kinetics Project No.____ Book No.____ 65

From Page No. 64

6/28/90 To transfected 293 cells. Change media to S.F. DMEM + F12 – Cys, – met media for 30 min.

Add same S.F. media w/ ^{35}S, met + Cys for 2 hrs.

Cells don't look great but continue anyway.

8 dishes x 2 ml each = 16 ml

Use 150 ml each Cys + met ^{35}S. 2 ml / dish

2 hr Pulse Label on 1 PM
off 6 3 PM – wash 2x refeed 2 ml PBS + Ca,Mg

Samples Chase

Time 0 hr (3 PM) Sup 2 ml (Label original)
Cells 1 ml 1% Triton + 5 mM Hepes

1 hr (4 PM) Sup 2 ml (PBS)
Cells 1 ml.

2 hr (5 PM) Sup 2 ml
Cell 1 ml

3 hr (6 PM) Sup 2 ml
Cell 1 ml

5 hr (8 PM) Sup 2 ml
Cell 1 ml

O.N. chase Sup 2 ml
Cell 1 ml

4 hr pulse – O.N. chase

Remove Sups. Wash 2x PBS
then lyse w/ Trix + Hepes

66

To Page No.____

Witnessed & Understood by me, [signature] Date 9/7/90

Invented by [signature] Date 8/6/90

Recorded by

Project No._____
Book No._____ TITLE Pulse chase of 405 Kinetics cont.

From Page No. 65

7/5/90 Immuno Precipitate the Supe and Cells collected

600 ml of Supe } 3 ml of RαH IgG 20'
300 ml of Cells }

↓

50 ml of Staph A cells (washed)

↓ wash

Resuspended in 40 ml SDS sample buffer

Run on 4-20% Gradient gels

Gel 1 Lanes)	1)	2 Hr Pulse	chase Hr-0	Supe	30 ml
	2)	"	Hr-0	Cells	
	3)	"	Hr-1	Supe	
	4)	"	Hr-1	Cells	
	5)	"	Hr-2	Supe	
	6)	"	Hr-2	Cells	
	7)	"	Hr-3	Supe	
	8)		Hr-3	Cells	
	9)	^{14}C MW Highs - 15 ml.			

Gel 2 Lanes)	1)	2 Hr Pulse	chase 5 Hr -	Supe	30 ml
	2)	"	5 Hr	Cells	
	3)	"	overnight chase -	Supe	
	4)	"	"	Cells	
	5)	4 Hr Pulse	0.4 chase	Supe	
	6)	"	"	Cells	
	7)	^{14}C Markers			

Results: See gel AutoRad in File (Kinetics of Ab secretion)

To Page No.____

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	9/7/90	[signature]	9/10/90
		Recorded by	

TITLE 293 Ha Var xfn #4, 5, 6 Project No. Book No. 67

From Page No. Sec p55 for xfn Key

6/27/90 xfn #4 Lb + Ha
xfn #5 Lc + Ha
xfn #6 Lc + Hc

Ha = 3.38 mg/ml
Hc = (5-C-4) 3 mg/ml
Lb = (3-6-8) 2 mg/ml
Lc = (3-C-7) 4.7 mg/ml
AdVA = 2.2 mg/ml

3 100 mm dishes / xfn = 30 ug DNA in 3 ml

xfn #	DNA	1/10 TE	CaCl2	2x Hepes
# 4	Ha - 4.5 ml Lb - 7.5 ml AdVA - 2 ml	1350 ml	150 ml	1.5 ml
# 5	Ha - 4.5 ml Lc - 3.2 ml AdVA - 2 ml	1.35 ml	150 ml	1.5 ml
# 6	Hc - 5 ml Lc - 3.2 AdVA - 2 ml	1.35 ml	150 ml	1.5 ml.

ppt on 5 pm
Shake 9:30 pm Feed w/ 10% FBS

6/28/90 Change media to S.F. DSOY + Insul.
6/29/90 AM Collected 30 ml of each xfn and purified by PEG
Desalted [redacted] into 3.5 ml PBS
Submitted to Bio Assay and Hu IgG Elisa.

To Page No.

Witnessed & Understood by me, Date 9/7/90
Invented by Date 8/6/90
Recorded by

Hu IgG Results

Project No._____

68 Book No._____ TITLE 405 Humanized Var #4, 5, 6

From Page No.__

7/5/90 Purified Ab submitted to Hu IgG Elisa

Hu Var #4, 5, 6 Prot A Purified 30 mL Exp #3.5.
i-PBS

ASSAY RESULTS ARE NOT CORRECTED FOR DILUTION

NO.	IDENTIFY EACH SAMPLE (GREEN AND/OR UNIQUE COMPONENTS)	DILUTION	ng/ml	NO.	IDENTIFY EACH SAMPLE (GREEN AND/OR UNIQUE COMPONENTS)	DILUTION
1	#4	1/10	>180	21		
2		1/100	55.3	22		
3		1/1000	16.6	23		
4	#5	1/10	>180	24		
5		1/100	31.6	25		
6		1/1000	8.5	26		
7	#6	1/10	>180	27		
8		1/100	40.7	28		
9		1/1000	6.0	29		
10				30		
11				31		

Results:
#4: 16.6 ug/ml
#5: 8.5 ug/ml
#6: 6.0 ug/ml

7/5/90 Gel of these Proteins.
Precipitated 1 ml of each above w/ 4 ml of Acetone.
Resuspended in ~25 ml SDS Gel Buffer
Run on 4-20% Gradient Gel.

(Lanes) 1) #4
2) #5
3) #6
4) Prestained BRL markers High (2ml diluted to 10 in buffer)
5) " Lows "

Results See Gel p71
#4 had some more degradation than others, but not too much
#5, 6 have v. little degradation.

To Page No.71

Witnessed & Understood by me: [signature] Date 9/7/90

Invented by [signature] Date 8/6/90

Recorded by

TITLE Bio Assay Results of 4D5 Humanized Versions #1, 2, 3

Project No.

Book No.

From Page No.

6/28/90

6/22/90 Assay

ard Curve

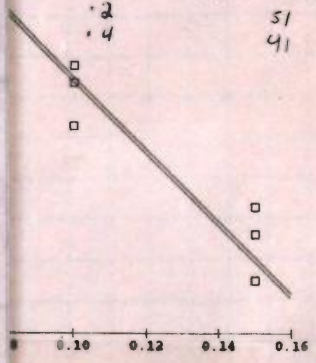
T 18R x 2C

4D5 Standard Curve for Her2 Assay 11568-69

0	4D5 Conc. (ug/ml)	1 OD (540 nm)	2 %Control
1	0.000	1.345	95.661451
2	0.000	1.497	106.472262
3	0.000	1.376	97.866287
4	0.025	1.223	86.984353
5	0.025	1.435	102.062589
6	0.025	1.278	90.896159
7	0.050	1.076	76.529161
8	0.050	1.195	84.992888
9	0.050	1.132	80.512091
10	0.075	0.990	70.412518
11	0.075	1.039	73.897582
12	0.075	1.096	77.951636
13	0.100	0.890	63.300142
14	0.100	0.965	68.634424
15	0.100	0.944	67.140825
16	0.150	0.699	49.715505
17	0.150	0.756	53.769559
18	0.150	0.790	56.187767

.2 51

.4 41



To Page No.

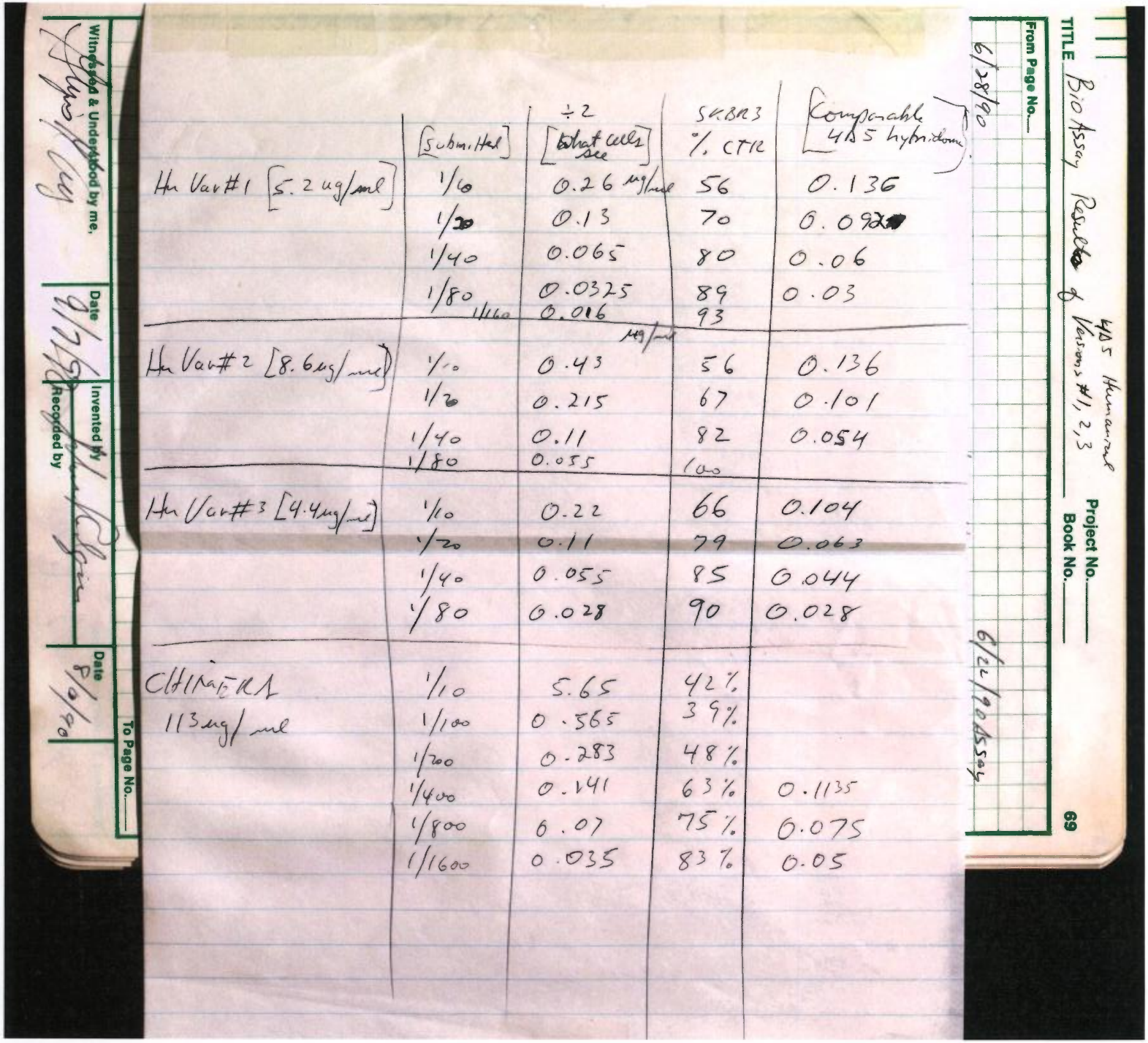
Witnessed & Understood by me,

Date 9/7/90

Invented by

Recorded by

Date 8/9/90



TITLE Bio Assay Results of 4D5 Humanized Versions #1, 2, 3
Project No.
Book No.
69
From Page No.
6/28/90
6/22/90 Assay

	[Submitted]	÷2 [what cells see]	SKBR3 % CTR	[Comparable 4D5 hybridoma]
Hu Var#1 [5.2 ug/ml]	1/10	0.26 ug/ml	56	0.136
	1/20	0.13	70	0.092
	1/40	0.065	80	0.06
	1/80	0.0325	89	0.03
	1/160	0.016	93	
Hu Var#2 [8.6 ug/ml]	1/10	0.43	56	0.136
	1/20	0.215	67	0.101
	1/40	0.11	82	0.054
	1/80	0.055	100	
Hu Var#3 [4.4 ug/ml]	1/10	0.22	66	0.104
	1/20	0.11	79	0.063
	1/40	0.055	85	0.044
	1/80	0.028	90	0.028
Chimeric 1 113 ug/ml	1/10	5.65	42%	
	1/100	0.565	39%	
	1/200	0.283	48%	
	1/400	0.141	63%	0.1135
	1/800	0.07	75%	0.075
	1/1600	0.035	83%	0.05

Witnessed & Understood by me. Date 9/19/90
Invented by
Recorded by Date 9/20/90
To Page No.

TITLE Bio Assay Results of 4D5 Humanized Versions #1, 2, 3

Project No.

Book No.

From Page No.

6/28/90

6/22/90 Assay

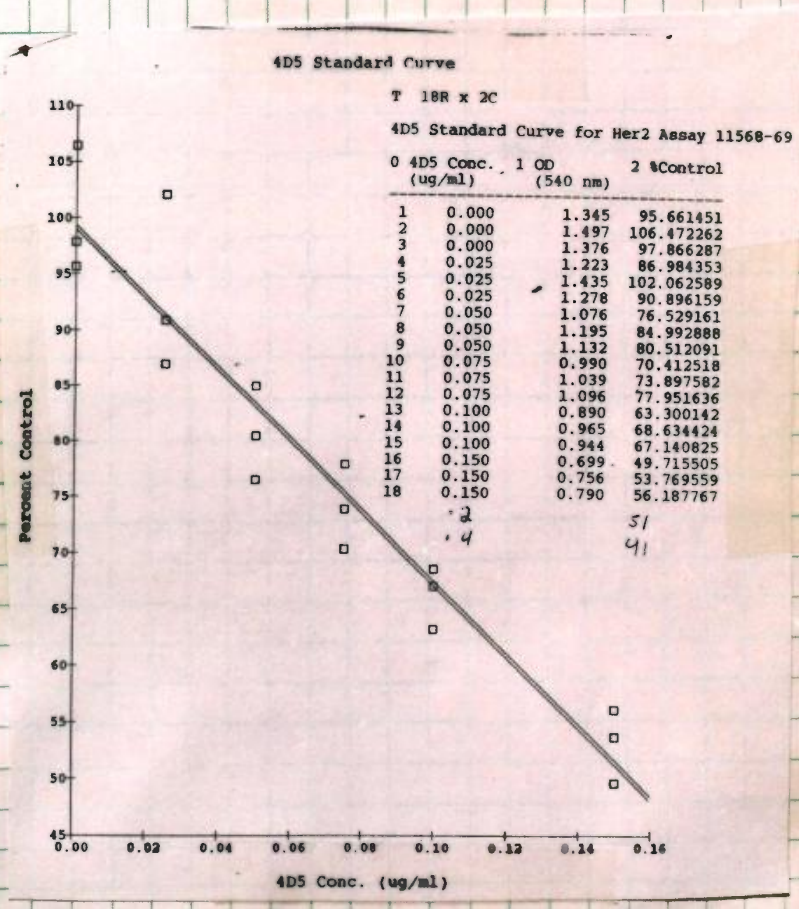
Ver #1 = W.T. humanized La + Ha

Ver #2 = La + Hb (A78(79)L)

Ver #3 = La + Hc (V102(109)Y)

Bio Assay Data

NO.	IDENTIFY EACH SAMPLE (ORDER AND/OR UNIQUE COMPONENTS)	DILUTION	% CONT.	ug/ml
1	Hu Var #1 (293)	1:10	56	.271
2	(5.2mg/ml)	1:20	70	.183
3		1:40	80	.120
4		1:80	89	.063
5		1:160	93	LTS
6		1:320	96	LTS
7	Hu Var #2 (293)	1:10	56	.271
8	(8.6mg/ml)	1:20	69	.202
9		1:40	82	.107
10		1:80	107	LTS
11		1:160	96	LTS
12		1:320	99	LTS
13	Hu Var #3 (293)	1:10	66	.208
14	4.4mg/ml	1:20	79	.136
15		1:40	85	.088
16		1:80	90	.056
17		1:160	94	LTS
18		1:320	96	LTS
19	4D5 2.16 stock	1:10	42	.845
20	113mg/ml	1:100	39	GTS
21		1:200	48	GTS
22		1:400	63	.297
23		1:800	75	.151
24		1:1600	83	.101
25				



0	4D5 Conc. (ug/ml)	1 OD (540 nm)	2 %Control
1	0.000	1.345	95.661451
2	0.000	1.497	106.472262
3	0.000	1.376	97.866287
4	0.025	1.223	86.984353
5	0.025	1.435	102.062589
6	0.025	1.278	90.896159
7	0.050	1.076	76.529161
8	0.050	1.195	84.992888
9	0.050	1.132	80.512091
10	0.075	0.990	70.412518
11	0.075	1.039	73.897582
12	0.075	1.096	77.951636
13	0.100	0.890	63.300142
14	0.100	0.965	68.634424
15	0.100	0.944	67.140825
16	0.150	0.699	49.715505
17	0.150	0.756	53.769559
18	0.150	0.790	56.187767
	.2		51
	.4		41

Sups are diluted 1:2 when added to SKBR3 cells.

So [4D5] read off Standard Curve is multiplied 2x For Comparison to Chart.

To Page No.

Witnessed & Understood by me, Date 9/7/90

Invented by

Recorded by Date 8/9/90

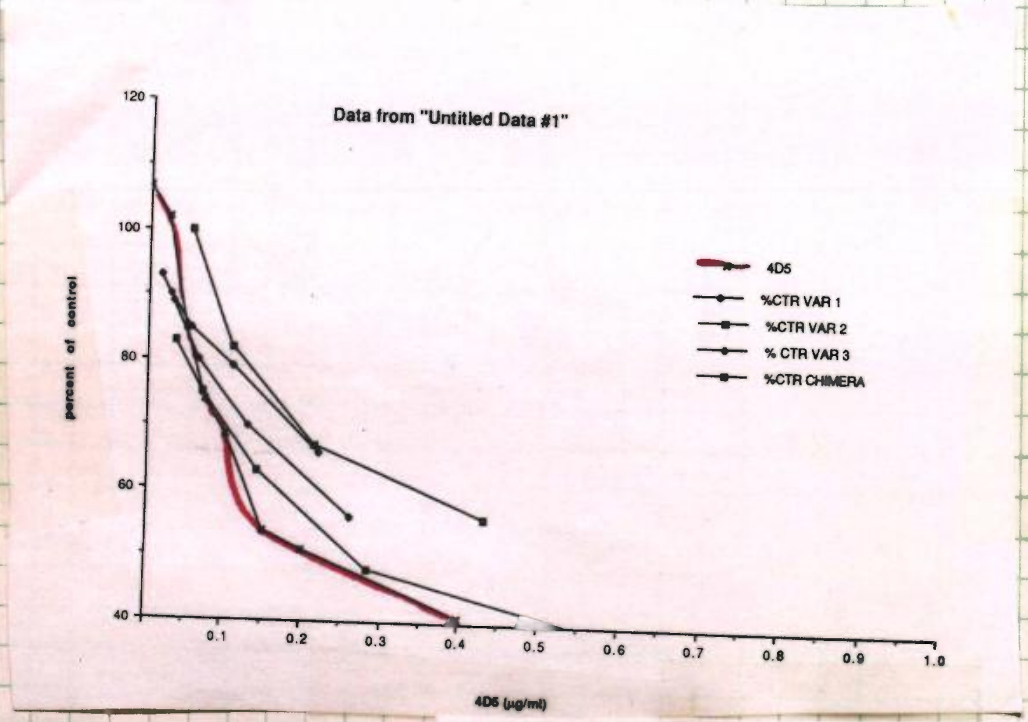
Project No.

70 Book No. TITLE Graph of BioAssay Data For Hu#1,2,3

From Page No. 69

6/29/90

From 6/22/90 Assay



Data from p 69

Variants of Humanized 4D5 (Expt # 310)

Heavy Chain

Version	Clone Name	Mutagenic Primer	Restriction Site Site Changes	Amino Acid Replacements
b	1/1	H7	removes PstI	A78(79)L
c	2/3	H8	None	V102(109)Y

Light Chain

Version	Clone Name	Mutagenic Primer	Restriction Site Site Changes	Amino Acid Replacements
b	3/2	L8	removes BglII	R66G
c	4/3	L7	removes XhoI	E55Y

CDR 3 1/3

The Kabat/Wu residue numbers are given first and then the absolute residue in brackets (only different for the heavy chain where there are insertions).

Interesting Combination of Light and Heavy Chains
(See Len Presta's rationale for why these variants were constructed)

Var	VL	VH	
1	a	a	"wild-type" humanized 4D5
2	a	b	
3	a	c	
4	b	a	
5	c	a	
6	c	c	

Witnessed & Understood by me, Date 9/7/90

Invented by Date 8/20/90

Recorded by

TITLE B.O Assay Results for Hu Var #4, 5, 6

Project No.

Book No.

From Page No.

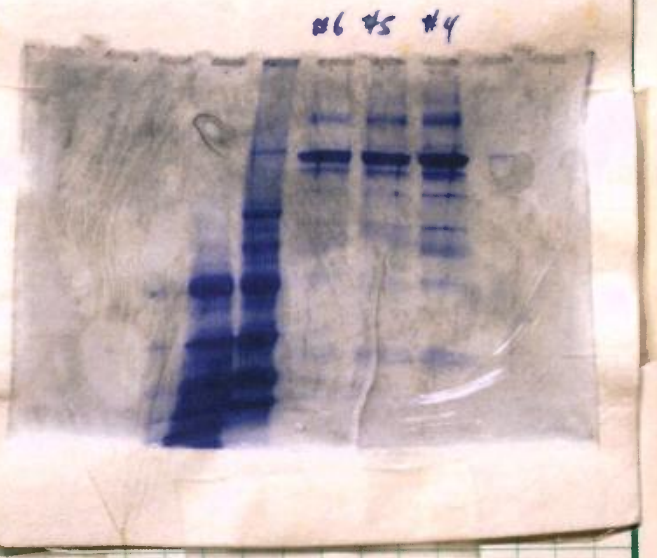
7/6/90

K. 7/6/90

See p 68

~~Hu IgG Std Curve Protein A~~

Protein Gel of Hu Var 4,5,6 PPT by Acetone



ASSAY RESULTS ARE NO

NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION	Conc ug/ml	% CTR
1	#4 16.6ug/ml	1/4	4.15	59
2		1/8	2.075	64
3		1/16	1.04	67
4		1/32	0.52	70
5	#5 8.5ug/ml	1/4	2.125	55.8
6		1/8	1.063	59
7		1/16	0.53	61
8		1/32	0.266	69.5
9	#6 6.0ug/ml	1/4	1.5	42
10		1/8	0.75	58.6
11		1/16	0.375	64.9
12		1/32	0.187	74.3

Data

Her2Bio#11568-88

7/5/90

4D5 Std Curve	MediaOnly	0.025 (ug/ml)	0.05	0.075	0.1	0.15	0.2	0.4	0.8	Ctrl
OD @ 540nm	1.149	1.088	0.929	0.944	0.707	0.651	0.554	0.581	0.51	0.797
	1.12	1.029	0.93	0.884	0.814	0.709	0.623	0.565	0.524	0.812
	1.187	1.035	0.925	0.902	0.838	0.668	0.659	0.623	0.532	0.822
Mean	1.152	1.051	0.928	0.910	0.786	0.676	0.612	0.590	0.522	0.810
SD	0.034	0.032	0.003	0.031	0.070	0.030	0.053	0.030	0.011	0.013
%Proliferation	100.0	91.2	80.6	79.0	68.3	58.7	53.1	51.2	45.3	70.3
		#4					#5		#6	
John Sample	1	2	3	4	5	6	7	8	9	10
	0.659	0.768	0.717	0.793	0.592	0.687	0.674	0.747	0.3	0.64
	0.683	0.71	0.813	0.787	0.66	0.651	0.707	0.811	0.501	0.665
	0.695	0.734	0.784	0.843	0.675	0.7	0.722	0.844	0.675	0.721
Mean	0.679	0.737	0.771	0.808	0.642	0.679	0.701	0.801	0.492	0.675
SD	0.018	0.029	0.049	0.031	0.044	0.025	0.025	0.049	0.188	0.041
%Proliferation	58.9	64.0	67.0	70.1	55.8	59.0	60.9	69.5	42.7	58.6
	#6									
John	11	12								
	0.623	0.757								
	0.627	0.729								
	0.71	0.758								
Mean	0.653	0.748								
SD	0.049	0.016								
%Proliferation	64.9	74.3								

To Page No.

Witnessed & Understood by me,

Date 9/5/90

Invented by

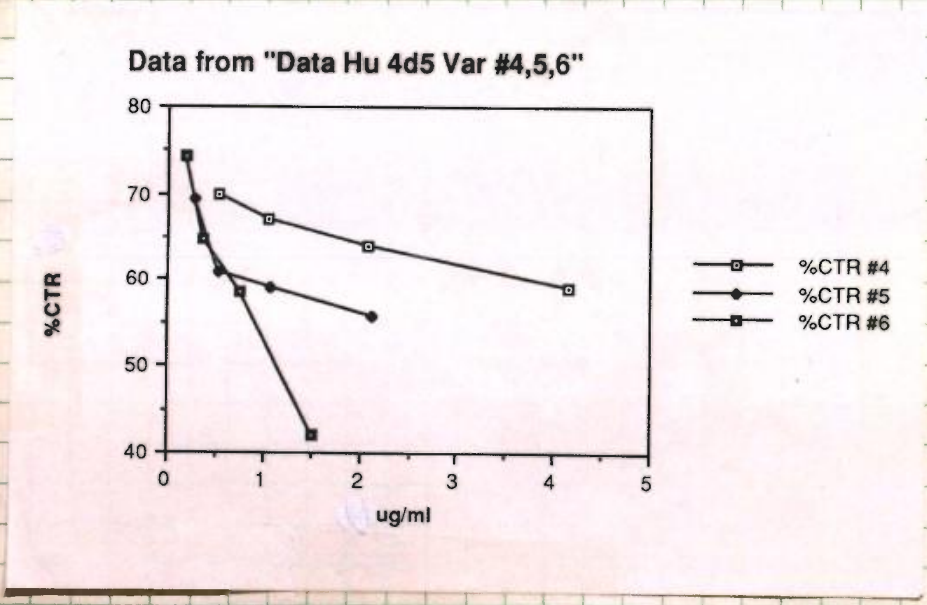
Recorded by

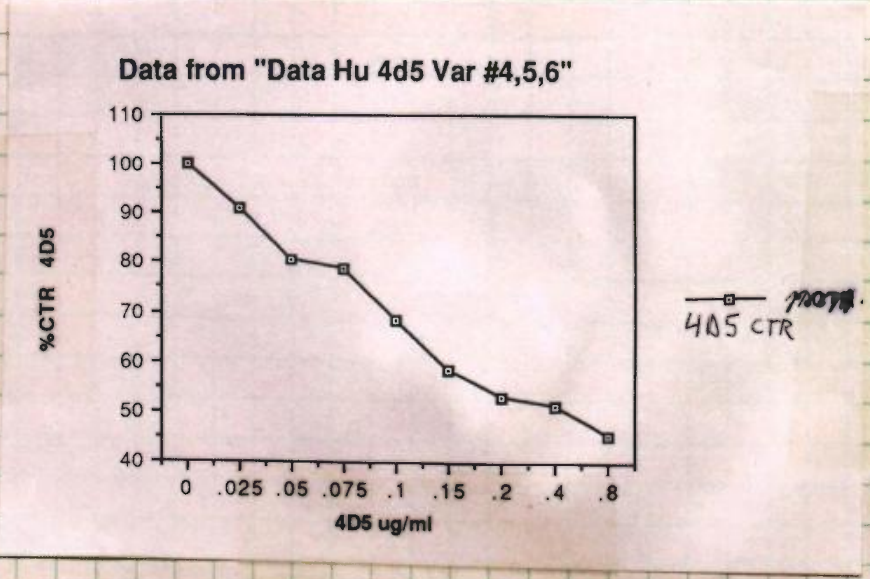
Date 8/6/90

Project No._____
Book No._____ TITLE BTO Assay Results Hu 405 Var #4,5,6

From Page No. 71

7/6/90





To Page No._____

Witnessed & Understood by me, Date 9/7/90
Invented by
Recorded by Date 9/10/90

TITLE Thaw 4D5 2.6 and 4D5 2.16 clones Project No. Book No. 73

From Page No.

For MTX Amplification repeat try.

7/9/90 A) Thawed 1 vial 4D5 2.16 From 5/15/90 in 1-100 mm dish 1% FBS + Gln.

B) Thawed 1 vial 4D5 2.6 From 5/10/90 1×10^7 cells into 1-100 mm dish 1% FBS + Gln

c) Also thawed 4D5 51.6 and 4D5 45.6 frozen 4/17 and 4/12 respectively.

Remember to refreeze some of each of these 4 clones back

7/10/90 2.6, 51, 45 are very confluent → split

7/11/90 Set up 2.6, 45.6, 51.6, 2.16 in 10, 15, 25 nM MTX

Media 1% XDZ + Gln (No GHT) 5×10^5 cells/100 mm dish

0.4 ml of 10,000 nM MTX in 400 ml = 10 nM MTX

0.6 ml " " " = 15 nM MTX

1.0 ml " " " = 25 nM MTX

Cell Counts: 1/40

2.6	19151	$= 7.66 \times 10^5$ c/ml	→ use 0.65 ml/dish
45.6	22700	$= 9 \times 10^5$ c/ml	→ use 0.55 ml/dish
51.6	27580	$= 1.1 \times 10^6$ c/ml	→ use 0.5 ml/dish
2.16	8426	$= 3.4 \times 10^5$ c/ml	→ use 1.4 ml/dish

7/12/90 Cells look O.K.

7/13/90 Cells 04 - Not fed

Froze cells 2 Vials each 2.6 2.45, 2.51 each vial = 1 100 mm² dish

To Page No. 74

Witnessed & Understood by me, Date 9/7/90

Invented by Date 7/12/90

Recorded by

Project No._____
Book No._____ TITLE MTX Amp of 4D5 Chimera

From Page No. 73

7/12/90 Stained 2.6, 45.6, 51.6 to see if their expression level is related to % of cells expressing Ab in whole population.

Results:
2.6 - all cells stain V. well.
45.6 ~~45.6~~ 1x - ~ 50% cells stain moderately.
51.6 - all cells stain moderately.

{Stained w/ 1° - Cappel R&H (Fig 6 H&L) 1/500 3hrs
2° Bacho P-S&R Fig 6 1/250 3hrs}

7/16 Fed Cells
7/17/90 - Cells not selecting - Not killing fast enough to prevent growth
Perhaps the levels of MTX are not ↑ enough,
Repeat w/ higher levels?

7/20/90 25nM is selecting some. Re-gen at 40, 60nM MTX

8/3/90 Clones of 51.6 in 25nM MTX - None in 40
Clones of 45.6 in 25, 40, 60 nM MTX.
Picked 50 of 45.6 from 60nM MTX

Typsinized rest from 60 MTX replated in 100, 250, 500 nM MTX
" " 40 MTX " " " "

To Page No. 84

Witnessed & Understood by me, [signature] Date 9/7/90
Invented by [signature]
Recorded by
Date 8/10/90

TITLE Hu 405 Variants 1-6 Concentrated. Project No. Book No. 75

From Page No.

7/6/90 Purified Abs in PBS at Conc of:

Pre Concentrated [ ]

1) 5 mg/ml
2) 8.6
3) 4.4
4) 16.6
5) 8.5
6) 6.0

Using the Centricon microconcentrators the volume was reduced ~4X

Centricon-10 used (10,000 mw cutoff).

Protein concentrations above (pre concentrated) were determined by IgG Elisa. This elisa was overestimating by ~50% because the Standard was off by ~50%. The new elisa is corrected by the Standard used is the chimeric 2.16 CHo. this was amino acid analyzed.

These 6 were submitted to the Hu IgG Elisa and 405 ECD binding assay.

7/6/90
Results: Var #1 - 12 mg/ml
" 2 - 15 mg/ml
" 3 - 9 mg/ml
" 4 - 31.4 mg/ml
" 5 - 7 mg/ml
" 6 - 8.8 mg/ml

Using the Chimeric 2.16 as Standard at 5.8 mg/ml by amino acid analysis Previously as 11.5 mg/ml by O.D. 280. This accounts for the protein conc of the Var not increasing 4X with Concentration By by 4X.

Same [ ] results for IgG and ECD Binding assays

Protein Gel of Hu 405 Ab - 10ul of each sample at above concentration
Lanes) 1 + Var #1
2 - Var #2
3 - Var #3
4 - Var #4
5 Var #5
6 Var #6
7 405 Chimeric - 10ul of 5.8 mg/ml.

To Page No.

Glycosylation 9/7/90 Recorded by Date 8/6/90

From Page No.

7/13/90 Submitted all 6 Hu 4D5 Variants & BioAssay w/ the Chimeric as well.

Concentrated material used as per p 77. ~~Obtained the~~ Protein Concentration determined by A_{280} Elisa using the Chimeric Standard newly analyzed by a.a. analysis.

Submitted to BioAssay at [ ] as follows:

1.6, 0.8, 0.4, 0.2, 0.15, 0.1, 0.05 ug/ml.

		Ab	diluent
CV=CV			
1.6(1)=12(x)	#1	133ul + 867 =	1.6 ug/ml
1.6(1)=15(x)	#2	107ul + 893 =	1.6 ug/ml
1.6(1)=9	#3	177 + 823 =	1.6 ug/ml
1.6/31.4	#4	51 + 949 =	1.6 ug/ml
1.6/7	#5	229 + 771 =	1.6 ug/ml
1.6/8.8	#6	182 + 818 =	1.6 ug/ml

Calculations used to get first dilution of 1.6 ug/ml

Samples submitted 1-49, in order of Var 1-6, Chimera in decending concentrations.
Sample #1 4D5 Var #1 1.6 ug/ml
#49 Chimeric 4D5 2.16, 0.05 ug/ml

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Witnessed & Understood by me, Date 9/7/90 Invented by Date 8/6/90
Recorded by

30' pulse → chase

Project No._____

TITLE 293 xfn of 405 the w.t. Vac for Kinetics labeling Book No._____ 77

From Page No.___

7/16/90 Feed cells at 9:AM (Split 7/5)

9 - 60 mm dishes for xfn

0.5 ml of DNA ppt x 9 = 4.5 ml of ppt at 10 ug/ml = 45 ug HCl ppt.

Ratio of ~ DNAs 5 : 5 : 2
H + L + AdVA

H chain (PA5) : 3.38 mg/ml
L chain (PA3) : 2.74 mg/ml
AdVA = 2.2 mg/ml

5/12 x 45 = 18.7 mg / 3.38 = 5.6 ml of PA5
18.7 mg / 2.74 = 6.8 ml of PA3
2/12 x 45 = 7.5 / 2.2 = 3.4 AdVA

DNA	1/10 TE	CaCl2	2x Hepes
5.6 ml of PA5 6.8 ml of PA3 3.4 ml of AdVA	2025 ml	225 ml	2.25 ml

ppt 4 min on 12:00
Shake at 4 pm.

To Page No.___

Witnessed & Understood by me, [signature] Date 9/7/90

Invented by [signature] Date 8/10/90

Recorded by [signature]

Project No._____
Book No._____ TITLE 293 pulse chase w/ thr 485 (w.T.)

From Page No. 77

7/17/90 TO transfected cells. Add ^{35}S met + Cys to dishes for 30 min. (Pulse)

8 dishes × 1.5 ml label = 12 ml label

No Pre-incubation w/ Cys⁻, met⁻ media.

12 ml label: F12, Dulbecco's w/o Cys, met + ^{35}S Cys, met.

100 uCi/ml Cys, met = 1200 uCi each CTR plate: 293 not xfn with Ab

label is 10 uCi/ml: so 120 ul of ^{35}S Cys + met each
Rinse 1× w/ PBS before adding label
Pulse 30 min – wash label off w/ PSO4 1×. Refeed w/ PSO4 5.0 ml.

11:35 Time 0 – take Sups + Cells (1 ml TNI × 1 w/ 1% + 5 units/ml Sups) p 67.2)
12:35 1 hr – Sup 5 mls + 1 ml Cells
1:35 2 hr ✓ ✓
2:35 3
3:35 4 hr – ✓ ✓
4:35 5 hr ✓ ✓
5:35 – 6 hr – ✓ ✓
10:AM – 23 hrs ✓ ✓ + CTR plate harvested at 23 hr Sup + Cells

7/18/90 Immune Precipitate Sups 1.5 ml and Cells 300 ml
(TO Compare w/ the last Kinetics exp p65 – need to get same proportion of Sups 6/20 of 5 ml = 1.5

– used 50:50 mix of RAH $I_gG(Fc)$ and RAH & Kappa chain – 4 ml / tube.

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Witnessed & Understood by me,	Date 9/7/90	Invented by	Date 8/10/90
		Recorded by	

From Page No.

7/17/90 Transient Transfection 293 cells w/ Hu 4A5 W.T. 3-100 dish
Collect Supe:

D.Ib 1) 24hrs, 48hrs 72hrs
2) 48hrs, 72hrs
3) 72hrs

100-3 dish = 3 ml ppt at 10ug/ml DNA = 30 ug DNA

Ratio 5 : 5 : 2
H - L - AdVA

H chain (pA5) : 3.38 ug/l
L chain (pA3) = 2.74 ug/l
AdVA = 2.2 "

DNA	4/10 TE	CaCl2	2x Hepes
	1350 ul	150 ml	1.5 mls
5 ml H chain (pA5)			
6 ml L chain (pA3)			
3 ml AdVA			
ppt on 1:30			

7/18/90 Am change media to all 3 plates to SF. + Transfection Cocktail
7/19/90 Cells still look fair ~90-95% Confluent, V. few floating.
Removed media from plate 1 - 0-24hrs - refed & Saved some
7/20/90 Cells looking more granular & dark, gaps appearing as cells clump. few floating.
media removed plate 1 - 24-48hrs - refed
plate 2 - 0-48hrs refed.
Purify Samples from 7/19-20/90 on Style A cells today.
7/21/90 Still few cells floating. though somewhat more unhealthy looking.
(Am) Removed media plate 1 - 48-72, plate 2 48-72, plate 3 - 0-72hrs. Refed
7/23/90 Kept media plate 1 72-120hrs.

To Page No. 80

Witnessed & Understood by me, Date 9/7/90 Invented by Date 8/6/90
Recorded by

From Page No. 29

7/24/90 Samples 1-6 as follows were purified on Staph A Gels on Saturd 7/21

Samples:
#1 Plate 1, 0-24hrs
#2 Plate 1, 24-48hrs
#3 plate 1, 48-72hrs
#4 plate 2, 0-48hrs
#5 plate 2, 48-72hrs
#6 plate 3, 0-72hrs.

Ohr is at start of Serum Free PBS 4 + Insulin. (Day after xfer)

Samples 10ml each.

A - 7th sample was taken on Monday 7/23

#7 plate 1, 72-120hrs

Samples ~~were~~ were purified on Staph A cells to 2.5mls, buffer exchanged on PD10 Sephadex G-25m Columns to PBS 3.5-4mls, then concentrated in Amicon-30 (30,000 mw cutoff) microconcentrators to ~900ul-1.0ml

7/25/90 Samples submitted to Ig & Elisa - for total protein.
ELISA binding - a whole Hu Ig+ std
b Hu Fab std
} comparison for degradation CHR

Conjugate
(a - Gt Hu Fc-HRP
b - Gt Hu Fab-HRP)

Submitted Samples:
Final Concentrated Samples 1-7 - diluted 1/100, 1/1000, 1/10,000
(5, 6, 7 ~~are~~ Purified + Conc from 9mls)

Sups prior to anything (purifying etc) 1-7 diluted 1/10 1/100, 1/1000
(1, 2, 3, 4 diluted 1/3 w/ 2x binding buffer) (5, 6, 7 - Sup straight from dish)

Sample #6 after buffer exchange / before concentration dil 1/100, 1/1000, 1/10^4
(4 mls total)

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Witnessed & Understood by me,	Date	Invented by	Date
[signature]	9/7/90	[signature]	8/10/90
		Recorded by:	

TITLE Kinetics - Pulse Chase of 4N3 the Ab

Project No.

Book No.

From Page No. 78

7/8/90 I.P. of Samples done. Resuspended in 35-40ml of SDS Sample buffer.

Run on 4-20% Gradient Gels. - 20ml/lane.

Gel 1 : Lane 1) T: 0hrs Exp
2) " Cells
3) T: 1hrs Exp
4) " Cells
5) T: 2hrs Exp
6) " Cells
7) Blank
8) ^{14}C MW markers
9) Prestained HMW markers

Gel 2 : Lane 1) T: 3hrs Exp
2) " Cells
3) T: 4hrs - Exp
4) " - Cells
5) T: 5hrs Exps
6) " Cells
7) Blanks
8)
9) ^{14}C MWM Lane
10) prestained HMW

Gel (3) Lane: 1) T: 6hrs Exps
2) " Cells
3) T: 23hrs Exps
4) " Cells
5) 2ys CTLs 23hrs Exps
6) " Cells
7) Blanks
8)
9) ^{14}C LMW markers
10) Prestained HMW markers

To Page No.

Witnessed & Understood by me, [signature] Date 9/7/90

Invented by [signature]

Recorded by

Date 8/10/90

Project No._____
Book No._____ TITLE Bio Assay Results Her-2

From Page No. 76

7/19/90 Concentrations of Ab (IgG) ~~Sent~~ submitted will be adjusted before calculating/working up data from bio assay.

IgG concentrations by elisa for the Human 4D5 Variants were determined with the chimeric 4D5 as the standard. This gives false readings (I think now) because the ~~the~~ values derived using ~~the~~ a humanized 4D5 as standard ~~are~~ are different.

The humanized 4D5 "wild type" made in E Coli is probably a better standard to use in determining the IgG Conc. of Humanized Ab's made in ~~CHO~~ ~~293~~ mammalian cells

Samples were submitted to Bio Assay (Her-2) on 7/3/90 based upon [IgG] using the 4D5 Chimera as standards. This Bio assay data will be used but adjusted for higher IgG concentrations.

Each dilution submitted will be multiplied by a factor ~~developed~~ developed ~~for the~~ from the [IgG] data on p83.

Var #1: 21.3/12.1 = 1.76 ∴ all dilutions for Var #1 multiplied by 1.76 before plotting [ ] to % CTR curve

Var #2 27/15.1 = 1.79

Var #3 15.3/9 = 1.7 Use Ave of 1.7 as factor to increase to correct conc

Var #4 5.5/3.2 = 1.72 Divide submitted conc by 2 before graphing as the true conc. seen by SKBR3 cells

Var #5 11.8/7.1 = 1.66

Var #6 14.2/8.8 = 1.61

To Page No.____

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	9/7/90	[signature]	8/10/90
		Recorded by	

TITLE Ig G Elisa of Hu 4D5 Var 1-6 w/ 2 Standards

Project No.

Book No.

From Page No.

7/19/90 Values for IgG Conc for Humanized 4D5 Variations 1-6 are higher using the Humanized Ab as Standard.

7-12-90

John Ridgway

(8.aaa)

date is ng/ml

	Chimeric Std. IgG1 Total Protein	Chimeric Std. HER2 ECD	Humanized Std. IgG1	Humanized Std. HER2 ECD
1	>100	>100	>100	>100
2	12.1	11.9	21.3	21.6
3	1.2	1.2	1.8	1.9
4	>100	>100	>100	>100
5	15.1	13.9	27.0	25.4
6	1.6	1.6	2.4	2.5
7	>100	73.7	>100	>100
8	9.0	8.7	15.3	15.5
9	0.8	0.8	1.2	1.4
10	>100	>100	>100	>100
11	31.4	30.2	64.9	59.0
12	3.2	3.4	5.5	5.6
13	82.6	59.5	>100	>100
14	7.1	6.6	11.8	11.3
15	0.7	0.7	1.0	1.2
16	>100	86.2	>100	>100
17	8.8	8.5	14.2	15
18	0.8	0.9	1.3	1.5

Key:

1	Hu 4D5 Var #1	1/100 dil
2	"	1/1000
3	"	1/10,000
4	Hu 4D5 Var #2	1/100 dil
5	"	1/1000
6	"	1/10,000
7	Hu 4D5 Var #3	1/100 dil
8	"	1/1000
9		1/10,000
10	Hu 4D5 Var #4	1/100 dil
11	"	1/1000
12	"	1/10,000
13	Hu 4D5 Var #5	1/100
14	"	1/1000
15	"	1/10,000
16	Hu 4D5 Var #6	1/100
17	"	1/1000
18	"	1/10,000

To Page No.

Witnessed & Understood by me, Date 9/7/90

Invented by John Ridgway Date 8/6/90

Recorded by

Project No._____
Book No._____

TITLE MTX Assay of ~~the HDS 6~~ CHO HDS chimera clones

From Page No. 74

7/20/90 Set up again 5×10⁵ cells/plate at 40 and 60nM MTX

Cell Counts:

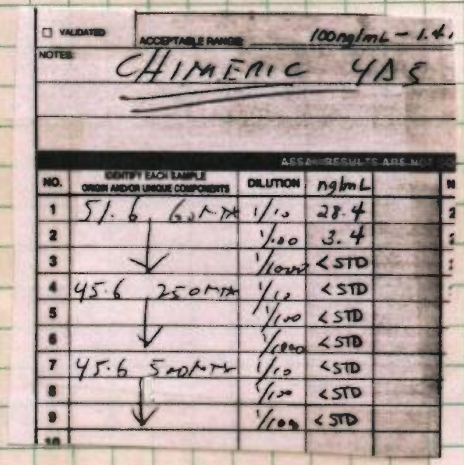
45.6 – 13,541 ×40 = 5.42×10⁵/ml – use 0.92ml/dish

51.6 – 10,726 ×40 = 4.29×10⁵/ml – use 1.17/dish

2.16 – 4458 ×40 = 178320/ml – use 2.8ml/dish.

8/9/90 45.6 Repletion of ~~clone~~ clones at 250nM MTX, 500nM MTX shows no IgG

51.6 clone in 60nM MTX <IgG.



☐ VALIDATED ACCEPTABLE RANGE 100ng/mL – 1.4.

NOTES: CHIMERIC HDS

NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION	ng/mL
1	51.6 60nM MTX	1/10	28.4
2	↓	1/100	3.4
3	↓	1/1000	< STD
4	45.6 250nM MTX	1/10	< STD
5	↓	1/100	< STD
6	↓	1/1000	< STD
7	45.6 500nM MTX	1/10	< STD
8	↓	1/100	< STD
9	↓	1/1000	< STD

To Page No._____

Witnessed & Understood by me, [signature] Date 9/7/90

Invented by [signature] Date 9/6/90

Recorded by

From Page No. 80

Hu 4105 w.T. 293 transient Exp

7/26/90 Total ug IgG

	IgG	0-24hrs	24-48hrs	48-72hrs	72hr total	No. 72-120hr
plate 1:	(post purify)	13.5ug +	12.7ug +	11.9ug =	38ug	38ug
(pre-purify)	Sups: (IgG)	18ug 25ug +	22.5ug 33 ug +	42ug 58ug =	82ug 116	yield 46% ave.

		0-24hrs	0-48hrs	48-72hrs	72hr total	
plate 2:	post purify	—	40ug	22ug =	62ug	
	Sups		63ug 64.5	30ug =	93	yield ~66%

		0-72hrs	
plate 3:	Purified + Conc	50ug total IgG	yield ~60%
	Sups	84ug total IgG	
plate 1	72-120hrs 37.5ug total purified / 45 Sups		— yield ~82%

% ECA binding IgG to total IgG as function of Age of Culture.

Q: Do the longer culture times (longer periods between ~~off~~ refeeding result in a greater % Ab not binding to ECA.

24hr culture	ECA	IgG	After Purification % ECA of IgG	Before Purification % ECA of total IgG
Plate 1 Sample 1 (0-24)	13.2	13.5	98%	~~89%~~ 72%
pl 1 Samp 2 (24-48)	11.2	12.7	88%	
pl 1 Samp 3 (48-72)	11.2	11.9	94%	~~65%~~ 76%
Pl 2 Sam 4 (0-48hr)	36.5	35.5	103%	~~80%~~ 75%
pl 2 Sam 5 (48-72)	17.7	18.1	97%	~~90%~~ 80%
Pl 3 Sample 6 (0-72hrs)	44.8	50.8	88%	66%
Pl 1 Sample 7 (72-120hrs)	36	45	80%	~~83%~~ 79%

To Page No.

Witnessed & Understood by me, Date 9/7/90

Invented by

Recorded by Date 9/10/90

Project No._____
Book No._____ TITLE Harvest Exp Data

From Page No. 85

7/26/90

1st 4D5 fab elisa
ECD Her 2 binding

ASSAY RESULTS ARE NOT CORRECTED FOR DILUTION

NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION	HER2 (HRP)	IgG (HRP)	NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION	HER2 (HRP)	IgG (HRP)
1	#1 pure/conc	1/100	>100	>100	21	#7 pure/conc	1/104	3.3	5.1
2	"	1/900	13.2	13.5	22	#1 ori Sup 1/2 dil	1/10	>100	>100
3	"	1/10000	1.1	1.5	23	↓	1/100	13.0	14.6
4	#2 pure/conc	1/100	>100	>100	24	↓	1/1000	1.2	1.9
5	↓	1/1000	11.2	12.7	25	#2 ori Sup 1/2 dil	1/10	>100	>100
6	↓	1/104	1.0	1.4	26	↓ (1 Hr shkd 1 cent)	1/100	17.0	17.4
7	#3 pure/conc	1/100	>100	>100	27	↓	1/1000	1.5	2.7
8	↓	1/1000	11.2	11.9	28	#3 ori Sup 1/2 dil	1/10	>100	>100
9	↓	1/104	[illegible]	[illegible]	29	↓ clumpy debris	1/100	30.8	34.2
10	#4 pure/conc	1/100	>100	>100	30	↓	1/1000	2.8	4.3
11	↓	1/10³	36.5	35.5	31	#4 ori Sup 1/2 dil	1/10	>100	>100
12	↓	1/10⁴	3.4	4.4	32	↓	1/100	35.1	43.8
13	#5 pure/conc	1/100	>100	>100	33	↓	1/1000	3.0	4.2
14	↓	1/10³	17.7	18.1	34	#5 Ori Sup neat	1/10	>100	>100
15	↓	1/104	1.5	2.8	35	↓	1/100	24.8	27.5
16	#6 pure/conc	1/100	>100	>100	36	↓	1/1000	2.3	3.4
17	↓	1/10³	46.5	45.6	37	#6 Ori Sup neat	1/10	>100	>100
18	↓	1/104	4.3	5.6	38	↓	1/100	59.5	85.1
19	#7 pure/conc	1/100	>100	>100	39	↓	1/1000	5.4	8.4
20	↓	1/1000	39.0	36.4	40	#7 Ori Sup neat	1/10	>100	>100
41	#7 Ori Sup neat	1/100	44.2	45.6					
42	↓	1/1000	4.0	6.0					
43	#6 bottle conc	1/100	89.3	>100					
44	Hals	1/1000	8.3	13.8					
45	↓	1/10,000	0.7	1.8					
46									

ng/ml results.

	M/ml
1	84.4
2	8.8
3	0.9
4	69.3
5	8.0
6	0.7
7	71.5
8	7.8
9	0.7
10	>100
11	23.1
12	2.5
13	99.0
14	11.7
15	1.1
16	>100
17	29.2
18	3.0
19	>100
20	24.8
21	2.5
22	69.3
23	9.0
24	0.9
25	>100
26	12.0
27	1.2
28	>100
29	20.8
30	2.4
31	>100
32	21.8
33	2.5
34	>100
	16.9

35	>100
36	39.0
37	4.0
38	>100
39	29.5
40	3.0
41	59.4
42	6.0
43	2.5
44	0.6
45	2.5
	2.5

Non Reducing
4-20% Grad prot Gel
of Samples 1-7 showing
intact Ab

To Page No._____

Witnessed & Understood by me,	Date	Invented by	Date
	9/12/90	Recorded by	8/6/90

TITLE Kd Analysis of 4D5 Hu Ab Project No. Book No. 87

From Page No.

8/8/90 Kd analysis by cell binding assay using SK-BR-3 cells and competition w/ ^{125}I 4D5 Ab.

Assay Done by Niels Dua

Control 4D5 hybridoma Ab : Kd 3.6 nM

4D5 Hu Var #1 ——— Kd 2.66 nM
4D5 Hu Var #4 ——— Kd 2.77 nM

To Page No.

Witnessed & Understood by me, Date 9/7/90 Invented by Date 8/10/90 Recorded by

Project No._____
Book No._____ TITLE: 293 Xfn of Hu Var 1-4

From Page No.__

7/31/90 4 100 plates each = 40ug DNA each Var + AdVA

	VL	VH
Var 1	pA3(a)	+ pA5(a)
Var 2	pA3(a)	+ Hb (5-b-4)
Var 3	pA3(a)	+ Hc (5-c-4)
Var 4	Lb (3-b-8)	+ pA5(a)

DNA's:
pA3 - Wild type Humanized L-chain 2.74ug/ml
pA5 - W.T Hu H-Chain 3.38ug/l
Hb (5-b-4) - Ver b of Hu H-Chain clone (5-
2.0ug/l
Hc (5-c-4) - Ver c of Hu H chain clone (5-c-4
3.0ug/l
Lb (3-b-8) - Ver b of Hu L-Chain clone (3-b-8
2.0ug/l

AdVA - 2.2ug/l

4 plates x 10ug DNA/plate = 40ug total in 4ml

	H : L : AdVA		H + L + AdVA
Ratio	5 : 5 : 2	→ for 40ug are	20 20 8ug

Var#	DNA	1/10 TE	2.5M CaCl2	2X Hepes
1	pA3 7.3ml pA5 6.0ml AdVA 4ml	1.8mls	200ul	2mls
2	pA3 - 7.3ml (5-b-4) Hb - 10ml AdVA - 4ml	1.8mls	200ul	2mls
3	pA3 - 7.3ml (5-c-4) Hc - 7ml AdVA - 4ml	1.8mls	200ul	2mls
4	(3-b-8) Lb - 10ml (pA5) Ha - 6.0ml AdVA - 4ml	1.8mls	200ul	2mls.

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Witnessed & Understood by me, Date 9/7/90
Invented by
Recorded by Date 8/10/90

From Page No 88

7/31/90 Cells refed at 9:30AM - Some plates have cells unevenly spaced.
Art DNA ave 1:4$ - 1:10
Have batch of 2X Hepes + 2.5M CaCl2
Let ppt go 7min.
#1 - 7.5min - more granular but O.K.
#2 - 7 - "
#3 - 4 - light ppt but O.K
#4 - 4min - light "

Glycerol Shock 5:30 - 6PM

8/1/90 Changed to PSOY + Tris + Gln (Serum Free)

8/3/90 Collected media 40mls ea Var 1-4. Refed with same.

8/5/90 Collected 40 ml from each Var 1-4 refed.

8/7/90 Collected 40 ml from each - discarded plates.

Supes were collected at 48hr. intervals over 6 days.

To Page No.

Witnessed & Understood by me: [signature] Date 9/7/90

Invented by [signature] Date 8/13/90

Recorded by

Project No._____
Book No._____ TITLE 293 Xfer of Hu Var 5-8

From Page No.__

8/1/90 4 plates 293 cells each - Fel 9AM 10% FBS.
Scaled light ~ 30-40% Confluent.

	VL		VH
Var 5	c (3-C-7) 4.7ug/l	+	a (PA5) 3.38ug/l
Var 6	c (3-C-7)	+	c (5-C-4) 3.0ug/l
Var 7	b (3-b-8) 2.0ug/l	+	e (PA13) 3.0ug/l clone 1/6 Paul Carter
Var 8	b (3-b-8)	+	d (PA14 clone 3/2) 0.16ug/l

AdVA 2.2ug/ml.
Prepare 4 ml each with ~ 10ug/ml total DNA each.
ea. L- 20ug / 8 H- 20ug AdVA 8ug

Var	DNAs	1/10 TE	2.5M CaCl2	2X HEPES
#5	3-C-7 - 5ml PA5 - 6.9ml AdVA - 4ml	1.8 mls	200 ml	2 mls
#6	3-C-7-(5ml) 5-C-4 (7.8ml) AdVA - 4ml.	1.8 mls	200 ml	2 mls
#7	3-b-8 - 11.7ml PA13 - 7.8ml AdVA - 4ml	1.8 ml	200 ml.	2 ml
#8	3-b-8 11.7ml PA14 150 ml AdVA 4ml	1.635 ml	200 ml	2 mls

To Page No.__

Witnessed & Understood by me, [signature] Date 9/7/90
Invented by [signature] Date 8/6/90
Recorded by [signature]

TITLE 293 Hrs of the Vols 5-8

Project No.

Book No.

91

From Page No.

8/1/90 Set up 50 Trans each

Set on 12:30 Shock 5-6pm Feed w/ 10% FBS

8/2/90 remove 10% FBS. Feed w/ PSG + Ins. Gln

8/4/90 Collected 40 ml each Vac 5-8.

8/6/90 Collected 40 ml each Vac 5-8

8/7/90 Collected 40 ml each Vac 5-8.

120 ml collected over 5 days for each Vac.

All samples purified on Protein A column and Concentrated by 30K mw cut off centricon tubes

To Page No.

Witnessed & Understood by me,

Date 9/7/90

Invented by

Recorded by

Date 8/10/90

Project No._____
Book No._____
TITLE Harvest + Purification of Hu Var 1-8.

From Page No.___

8/8/90 IgG Elisa submitted for Untreated sups of Var 1-8 and Final purified + Conc samples.

405 Hu Var 1-8 Final Yield:

		ug/ml IgG by Elisa		total ug IgG Protein.
#1 :	2.75mls	x 60.5 ug/ml	=	166 ug
#2 :	2.75mls	x 229 ug/ml	=	630 ug
#3 :	3.0mls	x 54.5 ug/ml	=	163 ug
#4 :	3.25mls	x 34 ug/ml	=	110 ug
#5 :	2.75mls	x 30.75 ug/ml	=	84 ug
#6 :	3.0mls	x 39 ug/ml	=	118 ug
#7 :	2.5mls	x 71.25 ug/ml	=	178 ug
#8 :	2.5mls	x 42 ug/ml	=	105 ug

Starting Sups 120ml for Var 1-8

				total Possible Protein	% Yield ~~total/total~~ x 100 =
#1 :	3.2ug/ml	X 120 mls	=	382	43%
#2 :	9 ug/ml	X 120 ml	=	1080	58%
#3 :	2.6ug/ml	X 120 ml	=	314	52%
#4 :	4.5 ug/ml	X 120 ml	=	544	20%
#5 :	2.3 ug/ml	X 120 ml	=	276	30%
#6 :	2.5 ug/ml	X 120 ml	=	306	38%
#7 :	2.75 ug/ml	X 120 ml	=	330	54%
#8 :	1.9 ug/ml	X 120	=	228	46%

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Witnessed & Understood by me, [signature] Date 9/5/90

Invented by [signature] Date 8/10/90

Recorded by

From Page No.____

8/8/90 Assay done 8/8/90

ASSAY RESULTS ARE NOT CORRECTED FOR DILUTION

NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION	ng/mL
1	Var 1 Pure + Conc	1/100	>STD
2		1/1000	54.2
3		1/10^4	6.7
4		1/10^5	<STD
5	Var 2 Pure + Conc	1/10^2	>STD
6		1/10^3	>STD
7		1/10^4	20.8
8		1/10^5	2.5
9	Var 3 Pure + Conc	1/10^2	>STD
10		1/10^3	44.5
11		1/10^4	6.5
12		1/10^5	<STD
13	Var 4 Pure + Conc	1/100^2	>STD
14		1/10^3	27.8
15		1/10^4	4.0
16		1/10^5	<STD
17	Var 5 Pure + Conc	1/10^2	>STD
18		1/10^3	25.5
19		1/10^4	3.6
20		1/10^5	<STD
21	Var 6 Pure + Conc	1/10^2	>STD
22		1/10^3	33.6
23		1/10^4	4.5
24		1/10^5	<STD
25	Var 7 Pure + Conc	1/10^2	>STD
26		1/10^3	64.5
27		1/10^4	7.8
28		1/10^5	<STD
29	Var 8 Pure + Conc	1/10^2	>STD
30		1/10^3	34.7
31		1/10^4	4.9
32		1/10^5	<STD
33	Var 1 Combined Sups	1/10	>STD
34		1/20	27.8
35		1/40	3.6
36	Var 2 Sup	1/10	>STD
37		1/100	91.1
38		1/1000	8.9
39	Var 3 Sup Combined	1/10	>STD
40		1/100	25.4

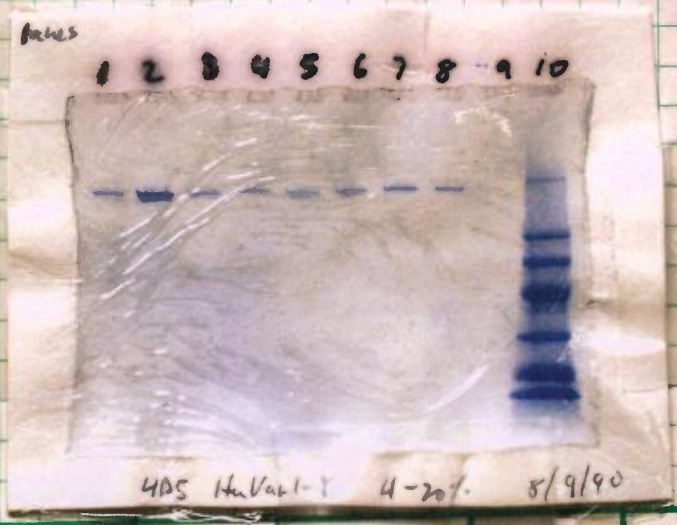
NO.	IDENTITY	DILUTION	ng/mL
41	Var 3 Sups	1/10^3	2.7
42	Var 4 Sups	1/10	>STD
43		1/10^2	39.7
44		1/10^3	5.1
45	Var 5 Sup	1/10	>STD
46		1/10^2	14.4
47		1/10^3	3.1
48	Var 6 Sup	1/10	>STD
49		1/20	16.9
50		1/100	3.4
51	Var 7 Sup	1/10	>STD
52		1/10^2	24.1
53		1/10^3	3.1
54	Var 8 Sup	1/10	>STD
55		1/10^2	16.9
56		1/10^3	2.1

Pure + Conc Samples are the final material.
Used Ave of 1/10^3 and 1/10^4 dilutions.

8/9/90 Sups are raw material as is off the dishes to calculate yield.

Run on 4-20% Gradient Gel No. 28.
10 ul of each Var.
15 ul of #5

Lanes: 1 Var 1
2 " 2
3 " 3
4 " 4
5 " 5
6 " 6
7 " 7
8 " 8
9 Blank
10 BRL High MW M



Witnessed & Understood by me, Date 9/7/90

Invented by Date 8/6/90

Recorded by

No.____

Project No._____
Book No._____ TITLE IgG ELISAs to understand Low Yield.

From Page No.__

8/7/90 Samples taken during the purification process of the 5 Var 1-8 are assayed to find which step the loss occurs.

ng/ml

ASSAY RESULTS ARE NOT CORRECTED FOR DILUTION

NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION	ng/ml	Corrected
1	Var 1 8/3 Sup	1/10	>100	>100
2		1/1000	30.5	24.5
3		1/10000	6.1	2.4
4	Var 1 8/3 elu	1/10	2.5	2.5
5	(by passed prot A)	1/100	2.5	2.5
6		1/1000	2.5	2.5
7	Var 1 8/3 Prot A elu	1/10	>100	>100
8	elu off prot A	1/100	>100	>100
9		1/1000	26.6	21.0
10	Var 1 8/3 2° elu	1/10	67.3	70.3
11	off Prot A	1/100	12.0	6.5
12		1/1000	0.5	0.6
13	Var 1 Sup from	1/10	2.5	2.5
14	filter conc	1/100	2.5	2.5
15		1/1000	2.5	2.5
16	Var 4 8/3 Sup	1/10	>100	>100
17		1/100	51.0	47.7
18		1/1000	9.6	4.5
19	Var 4 8/3 elu	1/10	0.9	6.0
20	Prot A bypass	1/100	2.5	0.6
21	Var 4 8/3 elu	1/1000	2.5	2.5
22	Var 4 8/5 Sup	1/10	>100	>100
23		1/100	44.2	39.9
24		1/1000	9.0	3.8
25	Var 4 8/5 elu	1/10	6.0	2.5
26	Prot A Bypass	1/100	2.5	2.5
27		1/1000	2.5	2.5
28	Var 4 8/7 Sup	1/10	>100	>100
29		1/100	41.0	36.8
30		1/1000	8.3	3.7
31	Var 4 8/7 elu	1/10	1.9	2.5
32	Prot A Bypass	1/100	2.5	2.5
33		1/1000	2.5	2.5
34	Var 4 S.p from	1/10	2.5	2.5
35	Filter Concentrator	1/100	2.5	2.5
36		1/1000	2.5	2.5
37	Sup Var 5 8/4	1/10	>100	>100
38		1/100	25.9	19.4
39		1/1000	4.9	1.8
40	Var 5 elu 8/4	1/10	3.4	2.2

ng/ml

NO	IDENTITY	DILUTION	ng/ml	Corrected
41	Var 5 elu 8/4	1/100	2.5	2.5
42		1/1000	2.5	2.5
43	Var 5 Sup 8/6	1/10	>100	>100
44		1/100	16.0	11.3
45		1/1000	2.8	1.3
46	Var 5 elu 8/6	1/10	2.5	2.5
47		1/100	2.5	2.5
48		1/1000	2.5	2.5
49	Var 5 Filter	1/10	2.5	2.5
50	Conc elu[Sup	1/100	2.5	2.5
51		1/1000	2.5	2.5
52	Var 2 Final Pure	1/100	>100	>100
53	8/7/90	$1/10^3$	>100	>100
54		$1/10^4$	26.8	19.5
55		$1/10^5$	5.4	1.9
56	Var 5 Final Pure	1/100	>100	>100
57	8/7/90	$1/10^3$	32.7	25.3
58		$1/10^4$	6.7	2.4
59		$1/10^5$	2.5	2.5
60				

Var #1 as example 8/3 collection of 40 ml
Raw sups: 2.4 ug/ml x 40 ml = 96 ug total IgG
all bound to prot A column (elu passthru 2.5)
Ab eluted off column 3 mls: 21 ug/ml x 3 ml = 63 ug total recovery,
the loss at this step is 35%. It is either not coming off the column or it is denatured by the low pH of the elution buffer so that it is not picked up by the elisa.

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Witnessed & Understood by me,	Date	Invented by	Date
Glyn H Cox	9/7/90	Recorded by	8/9/90

TITLE Gel of 2.16 Prebank Vs Un Banked

Project No.

Book No.

95

From Page No.

9/7/90 Ichlet Cells 35S

Runs Reduced + Non Reduced

Lanes

1) Non Banked Prod line - Cells
2) " - Supes
3) Prebanked - Cells
4) " - Supes
5) DP-12 - Cells CTRs
6) " - Supes

7) H mw stds

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Witnessed & Understood by me, Date 9/7/90

Invented by

Recorded by

Date 9/7/90

Project No._____
Book No._____ TITLE Stable 405 Hu Var 6 in 293 Cells

From Page No.__

9/25/90 pRK Hu 405 H+L chain. (one plasmid) Var#6 5-C-4-H
3-C-2-L

2.2mg/λ

HAMDR 0.25mg/λ

	DNA	1/10TE	CaCl2	2XHEPES
1. HAMDR only,	40 HAMDR	450ml	50ml	500ml
2. HAMDR + pRKVar6	5ml pRK Var 6 5ml HAMDR	440ml	50ml	500ml
3. pRKVar6 +AdVA.	5ml pRK Var 6 1ml AdVA	450	50ml	500ml
4) PA10 4/5 PA9 8	50ml PA9 56ml PA10 3AdVA	800ml	100ml	1ml

pH or 1:30

9/27/90 Split into 10% FBS + Colchicine range, 2ng–14ng/ml

10/2/90 all cells have died. Control 293 cells died at 2ng/ml level. Promoter on Hamdr plasmid not strong enough in 293 cells.

To Page No.__

Witnessed & Understood by me,	Date 10/2/90	Invented by	Date 10/5/90
		Recorded by	