

EXHIBIT 10

LABORATORY NOTEBOOK

No 723

AMGEN, INC.

LIN
EXHIBIT
114

AM 17 009822
CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER

FRITSCH v. LIN
INTER NOS 102,096
102,097, 102,334
LIN DOC NO L01136

Trial Exhibit 216
97-10814-WGY

Trial Exhibit ATS
97-10814-WGY

NOTEBOOK NO. 723

ISSUED TO Jean Egre

ON 4/17 1984

DEPARTMENT _____

RETURNED _____ 19 _____

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FRITSCH v. LTN
INTER NOS 102,096
102,097, 102,334
LTN DOC NO L011137

4/19/84 - 4/27/84

TITLE In Vivo Bioassay

bioassay

Project No. _____

Book No. _____

7

From Page No. _____

Passage of mEPO from CHO cells - Line 6 - dialyzed
 Same sample that was assayed in last bioassay, only at higher dilus so that all points
 will be on the standard curve

EXPERIMENT #884
 (Hypoxic) 4-23-84 - 4-27-84

Sample	Dose $\mu\text{g}/\text{dose}$	Volume
UE-37 (OPR)	1:2	6.52 \times 0.2ml
	1:4	4.42 \times 0.2ml
	1:8	2.22 \times 0.2ml
	1:8	1.12 \times 0.2ml
	1:16	0.52 \times 0.2ml
A 3-1-84 CHO - mEPO line 6	1:20	10 \times 0.2ml
	1:40	10 \times 0.2ml
	1:80	5 \times 0.2ml
	1:160	2.5 \times 0.2ml
PS-32 Input 73.4 μg total	1:2	2.1 \times 0.2ml
	1:2	1.1 \times 0.2ml
PS-32-4-1 51.1 μg total	1:2	2.5 \times 0.2ml
	1:4	2.5 \times 0.2ml
	1:4	1.25 \times 0.2ml
V-29 Input 14.56 μg total	1:2	2.4 \times 0.2ml
	1:2	1.2 \times 0.2ml
	1:4	0.6 \times 0.2ml
Bach Hyugen Urine 3-9-84 Dr. Powell		2 \times 0.2ml
0.5% BSA in physiol. saline		2 \times 0.2ml

Results ① Very encouraging - Monkey EPO produced in CHO cells has same calc. in vivo active units as estimated in RIA + in vitro bioassay. These assay results agree \pm one from last in vivo bioassay.
 ② Slope of the monkey EPO dose response is 11 to that of human std EPO. This was in question from the H₂ + L₂ data. Seemed as though the slope might have been different. But, we were always at the bottom of the dose-response curve.

③ Comparative Results for CHO Line 6

	units/ml
RIA -	(determinations)
in vitro bioassay -	(determinations)
in vivo bioassay -	42 \pm 5 (2 determinations)

FRITSCH v. LIN
 INTER NOS 102,096
 102,097, 102,334
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To Page No. _____

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		Recorded by _____	

1/19/84 - 412/84
 TITLE In Vivo Bioassay Label assay Project No. _____ Book No. _____ 7

From Page No. _____

Passage of cells from CCR cells - Luvle - analyzed
 Same sample that was assayed in last bioassay, only at higher dilus & not all points will be on the standard curve

EX-37 (OPB)	Dose $\mu\text{g}/\text{mouse}$	% ISE \hat{Y} U	CV O.E	Human Urinary Erythropoietin Secondary Standard	$b = S_b$	$S_{y \cdot x}$	n	r^2
1:2	0.2 \pm 0.2ml	22.7 \pm 2.1	370.8		41.4 \pm 2.8	6.0	25	0.95
1:4	0.4 \pm 0.2ml	31.5 \pm 1.4	380.4					
1:8	0.8 \pm 0.2ml	40.4 \pm 1.4	385.7					
1:16	1.6 \pm 0.2ml	52.7 \pm 1.4	460.1					
1:32	3.2 \pm 0.2ml	64.2 \pm 1.4	550.5					
A 3-11-84. CHO - MERO line 6	1:20	10 \pm 0.2ml	49.8 \pm 1.4	22.0	42 \pm 5.0	7.1	20	0.92
1:40	10 \pm 0.2ml	49.8 \pm 1.4	22.0					
1:80	10 \pm 0.2ml	49.8 \pm 1.4	22.0					
1:160	10 \pm 0.2ml	49.8 \pm 1.4	22.0					
PS-32 Input	1:2	10 \pm 0.2ml	42.3	0.15	347 \pm 6.3	60	15	0.94
PS-32-4-1	1:2	10 \pm 0.2ml	23.0	0.15				
1:4	10 \pm 0.2ml	23.0	0.15					
1:8	10 \pm 0.2ml	23.0	0.15					
1:16	10 \pm 0.2ml	23.0	0.15					
1-20 Input	1:2	10 \pm 0.2ml	31.7	0.15	514 \pm 6.1	5.5	14	0.92
1:4	10 \pm 0.2ml	31.7	0.15					
1:8	10 \pm 0.2ml	31.7	0.15					
1:16	10 \pm 0.2ml	31.7	0.15					
Beck H-pgon	Drain 3-9-84 Dr. Powell	2 \pm 0.2ml	12.0	0.05				
0.5% BSA in physiol. saline	2 \pm 0.2ml	1.0	1.02					

Results ① Very encouraging - Monkey EPO produced in CHO cells has same calc. in vivo as that units as estimated in RIA + in vitro bioassay. These assay results agree & are from last in vivo bioassay.
 ② Slope of the monkey EPO dose response is 11 to that of human std EPO. This was in question from the H₂ + L₂ data. Second as thought. The slope might have been different. But, we were placed at the bottom of the dose-response curve.

③ Comparative Results for CHO line 6
 Units/ml
 RIA - (determinative)
 in vitro bioassay - (determinative)
 in vitro bioassay - 42 \pm 5 (2 determinations)

FRITSCH v. LIN
 INTER NOS 102,096
 102,097, 102,334
 LIN DOC NO L01139

To Page No. _____

Submitted & Understood by me,	Date	Invented by	Date
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Project No. _____ 4/25/84 Competition RIA for EPO
 Book No. _____ TITLE _____

on Page No. _____

Samples:

CTO monkey EPO - line 7+10 combined. Used for mouse in vivo bio. effect expt. - injects into nodes
 3/31-4/6 - part of 3/15/84 continuation expt.
 Was 30 nM MTK amplification step → dialyzed o/n. $90 \pm 1.6 \text{ u/ml}$

CTO line 3 - Prior assays 3/13 - 39.7 u/ml Present value - 37.9 \pm 2.4 u/ml
 4/5 32.8 u/ml

MEPO - from 3/15/84 - 5 day collectn. - 10 nM MTK. Line 3 used for in vivo analyses of CTO material

CTO line 6 - Prior assays 3/13 - 40.2 u/ml Present value - 42.8 \pm 3.3 u/ml
 4/5 40.5 u/ml

MEPO. From 3/15/84 - 5 day collectn. - 10 nM MTK. Line 6 used for bioassay (in vitro + in vivo) of CTO material.

T8-30 Untreated (from stock tube, not P. 1:6.8 diln tube) $249.5 \pm 23 \text{ u/ml}$
 T8-30 Mock $334 \pm 27 \text{ u/ml}$
 T8-30 ENDO F treated $350.3 \pm 11 \text{ u/ml}$

Repeat from 4/15/84 expt. where only 1 point was near to being on the std curve
 this is the ENDO F expt. used for evaluation of in vivo + in vitro bioactivity of deglycosylated EPO.

Pure STD - from Porcine 100 u/ml by protein estimate
 Considered for use as std in RIA + in vitro + in vivo bioassay. $75.8 \pm 8.7 \text{ u/ml}$

Mouse sera #6 - had been injected c. CTO: EPO → tumor + high hematocrit.
 See injects. expt. - nude mice begun on 3/15/84
 Sera taken when the mouse was sacrificed. 297.5 u/ml

New line EPO - cos cell - 7 day collection - 4/10/84 $12.17 \pm 0.42 \text{ u/ml}$

Urine-KB
 Lot 82 - 50x conc. - Repeat from the last assay (4/10). when this sample was
 nearly off scale. Prior est = 320 u/ml $242.5 \pm 11.1 \text{ u/ml}$

Serum-free CTO expt.

If final purification of MEPO is to start, will need production in serum-free media
 & an estimate of the amt of EPO production/24 hr / 10^6 cells

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 INTER NOS 102,096
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Project No. _____
Book No. _____

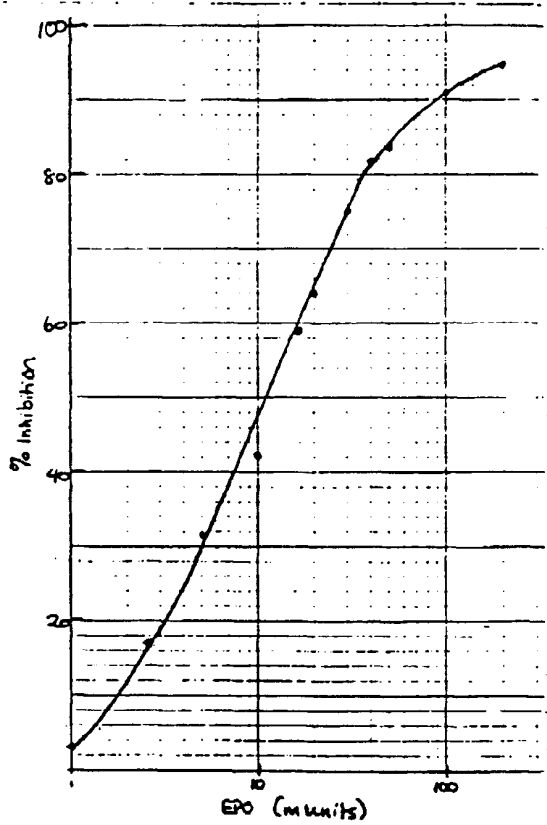
TITLE _____

From Page No. 8

Obtain samples from Ralph on 4/21/84. Flasks seeded & known # of cells in serum free media. Collection = 65 hr. post seeding
 0 nM MTX = 0.6 u/ml
 30 nM MTX = 2.4 u/ml
 100 nM MTX = 6.1 u/ml
 Use the flask ① series for the 3 determinations.

⑩ B7 sera - Kim patient sample - aplastic anemia.
 In 4/16/84 assay this sample was at the top of the scale + was estimated to be 50 u/ml. Repeat for a more accurate iter. 38.9 ± 5.3 u/ml

⑪ CHO: m EPO lines 7 + 10 combined 2/20 - 3/6/84 + dialyzed extensively overnight to remove traces of MTX. 27.1 ± 5.4 u/ml
 See 2. for their antibody m.v.s. effect experiments.



FRITSCH v. LIN
 INTER NOS 102,096
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 LIN DOC NO L01141

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Project No. _____
 Book No. _____ TITLE _____

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From Page No. 9

4/25/84 RIA
 5) Trasylol
 65) Mg. EC 204-3089 182 - 1:5000
 PBS 0.1% BSA - For total of 500 uL
 incubate 2 hrs. 37°
 STD = RIA STD #3

ED50 = 12.06
 Cool on ice
 Add 10) 125-EPO form 4/25/84 calculation.
 1396 cpm / 1 uL = 11446
 Incubate 0° overnight
 Process & 150 uL STOP A

#	SAMPLE	λ 165 + 0.1% BSA	cpm	% control	% inhibition	EPO/alg	EPO/Lml
1	STDs 0	430	2268	100%	0		
2	0	430	2194	223			
3	1ml = STD	435	2172	972	2.8		
4	25 uL = 12.5% D	420	1971	83.1	16.9		
5	5 uL = 2.5% C	430	1538	68.5	31.5		
6	10 uL = 5% C	425	1334	57.9	42.1		
7	15 uL = 7.5% C	425	916	40.7	59.3		
8	20 uL = 10% C	420	800	33.0	64		
9	30 uL = 15% C	415	626	24.8	75.2		
10	40 uL = 20% C	410	466	18.2	81.8		
11	50 uL = 25% C	405	447	16.4	83.5		
12	100 uL = 5% B	425	291	9.1	90.9		
13	200 uL = 10% B	420	210	5.3	94.7		
14	PF	430	953	97			
15	PF	430	99				
16	CHO-MEPO. 1 uL x 1000 20	410	906	37.9	62.1	18	72 u/ml
17	Myxobol 100 uL x 100	305	1106	47.3	52.7	11.5	92 u/ml
18	100 uL x 100	330	1651	72.8	27.2	9.5	90 u/ml
19	100 uL x 100	405	1948	56.8	13.2	2.2	88 u/ml
20	10	420	2109	94.3	5.7	1.2	120 u/ml
21	CHO. 1 uL x 3 1000	330	777	29.1	70.9	29	21 u/ml
22	50	380	928	38.9	61.1	17	34 u/ml
23	25	405	1246	57.8	46.2	9.4	37.6 u/ml
24	12.5	420	1592	70	30	5.0	40 u/ml
25	6.25	425	1852	83.4	16.4	2.5	40 u/ml
26	CHO-1 uL x 6 1000	330	718	29.1	70.9	29	24 u/ml
27	50	330	918	35.2	64.8	20	40 u/ml
28	25	405	1244	52.3	47.7	10	40 u/ml
29	12.5	420	1594	65	35	6	48 u/ml
30	6.25	425	1862	82.7	17.3	2.7	43.2 u/ml
31	TR-30. 100 uL x 1000	330	208	5.2	94.8	too high	
32	50	380	249	7.1	92.9	190	260 u/ml
33	20	400	330	13.3	86.7	61	203 u/ml
34	20	410	428	15.5	84.5	51	255 u/ml
35	10	420	656	26.2	73.8	28	280 u/ml
36	TR-30. 100 uL x 1000	330	442	16.2	83.8	49	333.2 u/ml
37	50	380	673	27	73	27	367 u/ml
38	25	405	848	35.2	64.8	50	
39	15	415	1360	59.2	40.8	70	344 u/ml
40	10	420	1658	73.2	26.8	93	292 u/ml
41	TR-30. 100 uL x 1000	330	36	1.2	98.8	67	455 u/ml
42	50	380	48	1.8	98.2	50	680 u/ml
43	25	405	1012	42.9	57.1	14	380 u/ml
44	15	415	1323	57.4	42.6	31	367 u/ml
45	10	420	1512	63.3	36.7	5.8	394 u/ml
46	Plus STD 1000	50	380	47.4	52.6	42	84 u/ml
47	25	405	798	32.9	67.1	21	84 u/ml
48	12.5	420	1260	55.4	44.6	9.9	71.2 u/ml
49	6.25	425	1760	75.6	24.4	4.0	64 u/ml

FRITSCH v. LIN
 INTR NOS 102,096
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#	SAMPLE	APC 12 66A	qpm	% Control	% Inhibition	EPO/Alig	EPO/ml
50	House B #6 CHO Tumor	25%	405	362	22.1	77.9	32 - too high
51	Inject mouse	0	420	907	38	62	15
52		5	425	948	39.9	60.1	16 } 32.0 u/ml } 297.5 u/ml
53		2	420	1540	67.6	32.4	5.5 } 27.5 u/ml
54	aliquot the CHO cells 1:10	25	355	336	11.2	88.8	80 } 10.7 u/ml
55	7 days	40	390	503	19	81	39 } 9.75 u/ml
56		20	410	786	32.3	67.7	21.5 } 0.75 u/ml } 10.17 ± 4.2 u/ml
57		10	420	1214	52.4	47.6	10 } 10 u/ml
58		5	425	1788	79.2	20.8	5.3 } 6.6 u/ml
59	Lot 22 Concentrate 1:100	50	380	262	7.7	92.3	120 } 240 u/ml
60	KB urine	25	405	390	13.8	86.2	60 } 240 u/ml
61		25	415	564	21.9	78.1	33 } 264 u/ml } 242.5 ± 11.1 u/ml
62		6	425	1009	42.7	57.3	14 } 233.3 u/ml
63		2	420	1620	71.3	28.7	47 } 255 u/ml
64	Polym. CHO - 6 star O ₂ Mtx	10	180	1163	49.9	50.1	12.1 } 0.484 u/ml } 0.6 u/ml
65	Serum free	100	330	1444	62.1	36.9	7.1 } 0.710 u/ml
66		30	400	2336	100	-	-
67		10	420	2326	100	-	-
68		3	425	2236	99.7	0.3	-
69	Polym. CHO - 6 star O ₂ Mtx	1:100	300	120	18.4	60.7	39.3 } 2.4 u/ml } 2.45 u/ml
70	Serum free	100	330	1885	83.8	16.2	2.5 } 2.5 u/ml
71		20	400	2260	101	-	-
72		10	420	2290	102	-	-
73		3	430	-	-	-	-
74	Polym. CHO - 6 star O ₂ Mtx	1:100	300	130	90.8	38	62 } 6 u/ml
75	Serum free	100	330	1458	63.8	36.2	7 } 7 u/ml } 6.1 ± 0.7 u/ml
76		20	400	2079	92.9	7.1	1.6 } 5.3 u/ml
77		10	420	2222	99.5	0.5	-
78		30	400	2375	107	-	-
79		10	420	2204	98.7	1.3	-
80	BT-KM in splash	1:100	300	180	336	11.2	88.8 } 80 } 32 u/ml
81	anemia sera	25	305	400	14.2	85.8	56 } 44.8 u/ml } 362.5 u/ml } 374.5 u/ml
82		10	380	854	35.5	64.5	20 } 40 u/ml
83		25	405	1442	63.0	37.0	7 } 28 u/ml
84	Neutrophils CHO Mtx prep urine	30	330	817	33.7	66.3	21.5 } 21.5 u/ml
85	for mouse inject	50	380	1184	51	49	11 } 220 u/ml } 221.5 u/ml
86	mouse 700 combined	25	405	1523	66.8	33.2	5.7 } 22.8 u/ml
87	the 2004	10	420	2014	89.8	10.2	1.75 } 175 u/ml

FRITSCH v. LIN
 INTER NOS 102,096
 102,097, 102,334
 LIN DOC NO 101143

To Page No. _____

Witnessed & Understood by me,	Date	Invented by	Date
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Effect of m.EPO in Balb/c mice

Line 8 C Balb/c mice from Charles River
 EPO: mEPO produced in CHO cells - 10ml LTY step - Dialyze overnight vs 2 changes of PBS - 2 liter each
 Line 7 & 8 combined 20 - 5/16/84 infection of CHO CCM
 Assay for EPO activity in 4/18/84 - 2215 5.4 U/ml
 Anticipate that all of RIA units are in vivo bioactive as well. But it is what was seen for the CHO produced mEPO - line 6 which should be a sample quite equivalent to this one.

Exptl Groups - House #	Treatment	Dose	U EPO/dose
• 77	+EPO	0.2ml 1:10 diln EPOmp	442U
• 17-79	+EPO	" "	442U
• 81	+EPO	0.5ml "	1105U
• 82	+EPO	0.2ml 1:1 diln EPOmp	442U
• 84	+EPO	" "	442U
• 85	Control	0.5ml 1:10 diln control	essentially 0
• 86	Control	0.2ml 1:1 diln control	only trace amount control by PBS
• 97	Control	" "	" "

Control - Control media (CCM) Sup of normal (m plasmaid) CHO cells, dialyzed in same manner as used for EPO containing CCM

Injecting Schedule - 5/11, 5/12, 5/13, 5/14, 5/17, 5/19, 5/11, 5/14, 5/16, 5/18, 5/21, 5/23, 5/25
 7/27, 7/30, 8/1, 8/4, 8/6, 8/25 PM @ 3:00 pm (0.3ml of 221 + 5.4 U/mouse)

Test Bloods - Retromental bleeds performed on M, W + F of each week to determine hematocrits

House #	Day 0	Day 2	Day 4	Day 7	Day 9	Day 11	Day 14	Day 16	Day 18
77	50.2	49.2	51.7	60.1	61.0	61.0	61.0	61.0	61.0
79	48.3	46.7	52.0	59.2	57.4	59.3	56.1	53.7	56.7
81	48.4	48.8	52.8	61.9	61.7	59.3	61.7	65.3	65.3
82	48.8	49.1	54.2	62.7	62.2	62.3	62.3	65.0	65.0
84	48.3	47.0	53.0	61.0	61.0	61.0	61.0	61.0	61.0
85	49.1	45.7	45.7	49.1	47.1	47.1	47.1	47.1	47.1
86	47.9	48.3	49.0	49.0	49.0	49.0	49.0	49.0	49.0
97	51.2	52.1	52.5	52.5	52.5	52.5	52.5	52.5	52.5
Ave Control	49.4	50.2	49.7	49.6	49.7	49.7	49.7	49.7	49.7

Mouse #	Day 21	Day 23	Day 25	Day 27	Day 29	Day 32	Day 35	Day 37	Day 39
77	57.6	58.0	57.3	57.3	57.3	57.3	57.3	57.3	57.3
79	51.1	57.0	56.3	56.3	56.3	56.3	56.3	56.3	56.3
81	60.7	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
82	60.0	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5
84	61.1	61.1	61.1	61.1	61.1	61.1	61.1	61.1	61.1
85	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
86	51.1	51.1	51.1	51.1	51.1	51.1	51.1	51.1	51.1
97	51.1	51.1	51.1	51.1	51.1	51.1	51.1	51.1	51.1

FRITSCH v. LIN 102,097, 102,334
 INTER NOS 102,096 LIN DOC NO L01144

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	Day 45	Day 46	Day 47	Day 48	Day 49	Day 50	Day 51	Day 52	Day 53	Day 54	Day 55	Day 56	Day 57	Day 58	Day 59	Day 60
184	7/15/04	7/16/04	7/17/04	7/18/04	7/19/04	7/20/04	7/21/04	7/22/04	7/23/04	7/24/04	7/25/04	7/26/04	7/27/04	7/28/04	7/29/04	7/30/04
91.4	27/50=37.9	27/55=38.2	27/60=38.5	27/65=38.8	27/70=39.1	27/75=39.4	27/80=39.7	27/85=40.0	27/90=40.3	27/95=40.6	28/00=40.9	28/05=41.2	28/10=41.5	28/15=41.8	28/20=42.1	28/25=42.4
80.46	25/50=31.2	25/55=31.5	25/60=31.8	25/65=32.1	25/70=32.4	25/75=32.7	25/80=33.0	25/85=33.3	25/90=33.6	25/95=33.9	26/00=34.2	26/05=34.5	26/10=34.8	26/15=35.1	26/20=35.4	26/25=35.7
80.91	25/50=31.7	25/55=32.0	25/60=32.3	25/65=32.6	25/70=32.9	25/75=33.2	25/80=33.5	25/85=33.8	25/90=34.1	25/95=34.4	26/00=34.7	26/05=35.0	26/10=35.3	26/15=35.6	26/20=35.9	26/25=36.2
98.9	27/50=37.9	27/55=38.2	27/60=38.5	27/65=38.8	27/70=39.1	27/75=39.4	27/80=39.7	27/85=40.0	27/90=40.3	27/95=40.6	28/00=40.9	28/05=41.2	28/10=41.5	28/15=41.8	28/20=42.1	28/25=42.4
48.5	27/50=37.9	27/55=38.2	27/60=38.5	27/65=38.8	27/70=39.1	27/75=39.4	27/80=39.7	27/85=40.0	27/90=40.3	27/95=40.6	28/00=40.9	28/05=41.2	28/10=41.5	28/15=41.8	28/20=42.1	28/25=42.4
152.5	27/50=37.9	27/55=38.2	27/60=38.5	27/65=38.8	27/70=39.1	27/75=39.4	27/80=39.7	27/85=40.0	27/90=40.3	27/95=40.6	28/00=40.9	28/05=41.2	28/10=41.5	28/15=41.8	28/20=42.1	28/25=42.4
3	48.1	48.3	48.9	48.6	47.8	46.3	47.4	45.5	46.5							
66	Day 49	Day 71	Day 73	Day 76	Day 78	Day 80	Day 85	Day 85	Day 86							
184	7/19/04	7/11/04	7/13/04	7/16/04	7/18/04	7/20/04	7/23/04	7/25/04	7/27/04							
30.1	27/50=37.2	27/55=36.6	27/60=35.8	27/65=34.9	27/70=34.1	27/75=33.2	27/80=32.3	27/85=31.4	27/90=30.5							
48.3	27/50=37.2	27/55=36.6	27/60=35.8	27/65=34.9	27/70=34.1	27/75=33.2	27/80=32.3	27/85=31.4	27/90=30.5							
39.3	27/50=37.2	27/55=36.6	27/60=35.8	27/65=34.9	27/70=34.1	27/75=33.2	27/80=32.3	27/85=31.4	27/90=30.5							
47.5	27/50=37.2	27/55=36.6	27/60=35.8	27/65=34.9	27/70=34.1	27/75=33.2	27/80=32.3	27/85=31.4	27/90=30.5							
40.7	27/50=37.2	27/55=36.6	27/60=35.8	27/65=34.9	27/70=34.1	27/75=33.2	27/80=32.3	27/85=31.4	27/90=30.5							
5=49.6	27/50=37.2	27/55=36.6	27/60=35.8	27/65=34.9	27/70=34.1	27/75=33.2	27/80=32.3	27/85=31.4	27/90=30.5							
35=48.3	27/50=37.2	27/55=36.6	27/60=35.8	27/65=34.9	27/70=34.1	27/75=33.2	27/80=32.3	27/85=31.4	27/90=30.5							
52.6	27/50=37.2	27/55=36.6	27/60=35.8	27/65=34.9	27/70=34.1	27/75=33.2	27/80=32.3	27/85=31.4	27/90=30.5							
2	50.9	49.7	49.2	47.6	47.9	48.9	46.7	46.1	46.1							
98	Day 93															
84	8/1/04															
46.6																
45.8																
42.9																
42.5																
44.1																
47.9																
47.5																

FRITSCH v. LIN
 INTER NOS 102,096
 102,097, 102,334
 LIN DOC NO L01145

AM 17 009831
 CONFIDENTIAL
 SUBJECT TO PROTECTIVE ORDER

EFFECT of EPO on BALB C MICE
 Summary of Hematocrit data

Average of 2

Low

High (Control)

Average of 3

	Average of 2	Low	High (Control)	Average of 3
1	49.6	49.4	49.5	49.5
2	47.9	47.8	47.8	47.9
3	48.5	48.5	48.5	48.5
4	49.9	49.8	49.8	49.7
5	47.5	47.5	47.5	47.6
6	42.8	42.8	42.8	42.9
7	45.4	45.4	45.4	46
8	47.6	47.6	47.6	48.4
9	48.1	48.1	48.1	49.7
10	47.5	47.5	47.5	49.4
11	47.5	47.5	47.5	49.7
12	47.5	47.5	47.5	49.7
13	47.5	47.5	47.5	49.7
14	47.5	47.5	47.5	49.7
15	47.5	47.5	47.5	49.7
16	47.5	47.5	47.5	49.7
17	47.5	47.5	47.5	49.7
18	47.5	47.5	47.5	49.7
19	47.5	47.5	47.5	49.7
20	47.5	47.5	47.5	49.7
21	47.5	47.5	47.5	49.7
22	47.5	47.5	47.5	49.7
23	47.5	47.5	47.5	49.7
24	47.5	47.5	47.5	49.7
25	47.5	47.5	47.5	49.7
26	47.5	47.5	47.5	49.7
27	47.5	47.5	47.5	49.7
28	47.5	47.5	47.5	49.7
29	47.5	47.5	47.5	49.7
30	47.5	47.5	47.5	49.7
31	47.5	47.5	47.5	49.7
32	47.5	47.5	47.5	49.7
33	47.5	47.5	47.5	49.7
34	47.5	47.5	47.5	49.7
35	47.5	47.5	47.5	49.7
36	47.5	47.5	47.5	49.7
37	47.5	47.5	47.5	49.7
38	47.5	47.5	47.5	49.7
39	47.5	47.5	47.5	49.7
40	47.5	47.5	47.5	49.7
41	47.5	47.5	47.5	49.7
42	47.5	47.5	47.5	49.7
43	47.5	47.5	47.5	49.7
44	47.5	47.5	47.5	49.7
45	47.5	47.5	47.5	49.7
46	47.5	47.5	47.5	49.7
47	47.5	47.5	47.5	49.7
48	47.5	47.5	47.5	49.7
49	47.5	47.5	47.5	49.7
50	47.5	47.5	47.5	49.7
51	47.5	47.5	47.5	49.7
52	47.5	47.5	47.5	49.7
53	47.5	47.5	47.5	49.7
54	47.5	47.5	47.5	49.7
55	47.5	47.5	47.5	49.7
56	47.5	47.5	47.5	49.7
57	47.5	47.5	47.5	49.7
58	47.5	47.5	47.5	49.7
59	47.5	47.5	47.5	49.7
60	47.5	47.5	47.5	49.7
61	47.5	47.5	47.5	49.7
62	47.5	47.5	47.5	49.7
63	47.5	47.5	47.5	49.7
64	47.5	47.5	47.5	49.7
65	47.5	47.5	47.5	49.7
66	47.5	47.5	47.5	49.7
67	47.5	47.5	47.5	49.7
68	47.5	47.5	47.5	49.7
69	47.5	47.5	47.5	49.7
70	47.5	47.5	47.5	49.7
71	47.5	47.5	47.5	49.7
72	47.5	47.5	47.5	49.7
73	47.5	47.5	47.5	49.7
74	47.5	47.5	47.5	49.7
75	47.5	47.5	47.5	49.7
76	47.5	47.5	47.5	49.7
77	47.5	47.5	47.5	49.7
78	47.5	47.5	47.5	49.7
79	47.5	47.5	47.5	49.7
80	47.5	47.5	47.5	49.7
81	47.5	47.5	47.5	49.7
82	47.5	47.5	47.5	49.7
83	47.5	47.5	47.5	49.7
84	47.5	47.5	47.5	49.7
85	47.5	47.5	47.5	49.7
86	47.5	47.5	47.5	49.7
87	47.5	47.5	47.5	49.7
88	47.5	47.5	47.5	49.7
89	47.5	47.5	47.5	49.7
90	47.5	47.5	47.5	49.7
91	47.5	47.5	47.5	49.7
92	47.5	47.5	47.5	49.7
93	47.5	47.5	47.5	49.7
94	47.5	47.5	47.5	49.7
95	47.5	47.5	47.5	49.7
96	47.5	47.5	47.5	49.7
97	47.5	47.5	47.5	49.7
98	47.5	47.5	47.5	49.7
99	47.5	47.5	47.5	49.7
100	47.5	47.5	47.5	49.7

AM 17 009832
 CONFIDENTIAL
 SUBJECT TO PROTECTIVE ORDER

FRIESEN V. LIN
 INTER NDS 102,096
 102,097, 102,334
 LIN DOC NO L01146

CHO LINES 340 Fed. EPO in dialyzed CHO
collected 2/20 2x P
Yellow Control dialyzed CHO

EFFECT OF mEPO IN BALB C MICE 5/29

EXPTL Group	HOUSE #	TREATMENT	DOSE
	118	+ EPO	0.4 Red IP
	119	+ EPO	0.4 Red IP
	120	CONTROL	0.4 Yellow IP
	121	CONTROL	0.4 Yellow IP

CONTROL - dialyzed CHO (Yellow)

INJECTION SCHEDULE 5/25, 5/26, 5/27, 6/1, 6/4, 6/5

TEST BLOODS

PREBLEED	DAY 0	Day 5	Day 6	Day 8	Day 10	Day 12	Day 15	Day 17	Day 20
	5/26/04	6/1/04	6/4/04	6/6/04	6/16/04	6/11/04	6/21/04	6/15/04	6/18/04
118	47.7	53.9	61.0	63.4	67.2	66	62	59.1	53
119	47.5	52.9	62.3	61.9	65.5	66	62	59.1	53
120	59.9	58.2	49.2	49.2	48.3	49.5	48	46	44
121	47.4	42.6	44.2	48.2	50.1	47.4	49.3	47.3	48

Sacrifice #118 on 6/19/04 for autopsy. Remove portions of spleen, liver, kidney, stomach, and thymus. Fix in Bouin's fluid. Fix in Bouin's fluid. Fix in Bouin's fluid.

Take hemato-crit on all: (1) CHO-EPO-IP = 41, 46; 39.5, 35.2 (2) CHO-CCM-IP = 46, 47.4; 43.1, 44.8; 41.3 (3) CHO-IP = 27.8, 24.7; 46.5, 40.3

Day 22	Day 24	Day 27	Day 29	Day 31	Day 34	Day 36	Day 39	Day 41	Day 43
6/20/04	6/22/04	6/25/04	6/27/04	6/29/04	7/2/04	7/6/04	7/19/04	7/11/04	7/13/04
47.7	51.0	50	48.3	46	46.6	45.7	48.3	45.9	47
50	45.5	49.2	46.3	46.8	46	48.3	50	48.8	46
48.3	42.8	44.6	45	45.6	45.6	46	48.3	48.3	47

IRTSCH v. LIN
INFR NOS: 102,096
102,097, 102,334
LIN DOC NO L01147

AM 17 009833
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46	Jan 49	May 50	Nov 50	May 51	Nov 51	May 52	Nov 52	May 53	Nov 53
16	181	7/18/84	5/10/84	3/29/84	2/29/84	2/29/84	2/29/84	2/29/84	2/29/84
59	375	2/27/85	3/10/85	2/27/85	2/27/85	2/27/85	2/27/85	2/27/85	2/27/85
59	SD	2/27/85	2/27/85	2/27/85	2/27/85	2/27/85	2/27/85	2/27/85	2/27/85
66	487	2/27/85	2/27/85	2/27/85	2/27/85	2/27/85	2/27/85	2/27/85	2/27/85
66	487	2/27/85	2/27/85	2/27/85	2/27/85	2/27/85	2/27/85	2/27/85	2/27/85

FRITZSCH V. LIN
 INTER NOS 102,096
 102,097, 102,334
 LIN DDC NO L01148

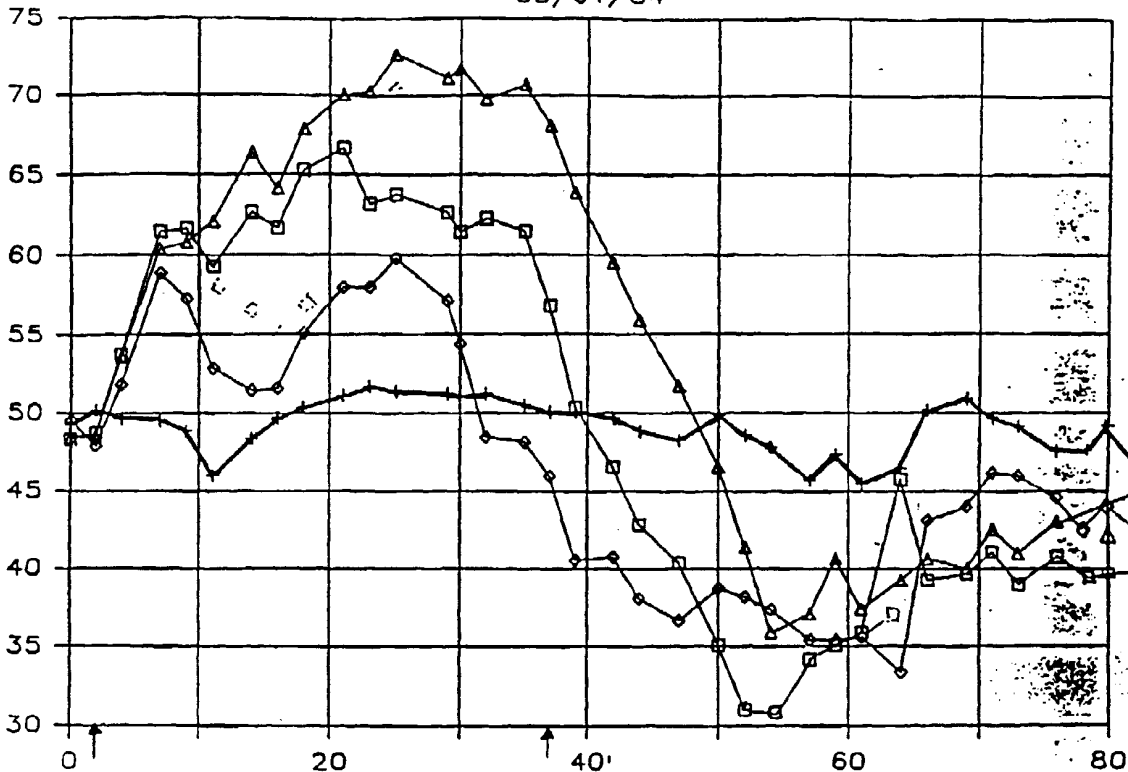
AM 17 009834
 CONFIDENTIAL
 SUBJECT TO PROTECTIVE ORDER

Project No. _____
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 TITLE _____
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EFFECT OF MONKEY EPO ON BALBC MICE

05/01/84

HEMATOCRIT (%)



FRITTSCH v. LIN 102,037, 102,134
 INTL. NO. 102,036 LIN DR. NO. 101457

□ 81 + 11 u EPO + CONT AVG + CONTROL MEDIA ◇ 77-79 AVG + 4.4 u EPO △ 82-84 AVG + 44 u EPO

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

AM 17 009835
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 SUBJECT TO PROTECTIVE ORDER

LE | 5/23-5/24 - EPO Competition RIA

Project No. _____

Book No. _____

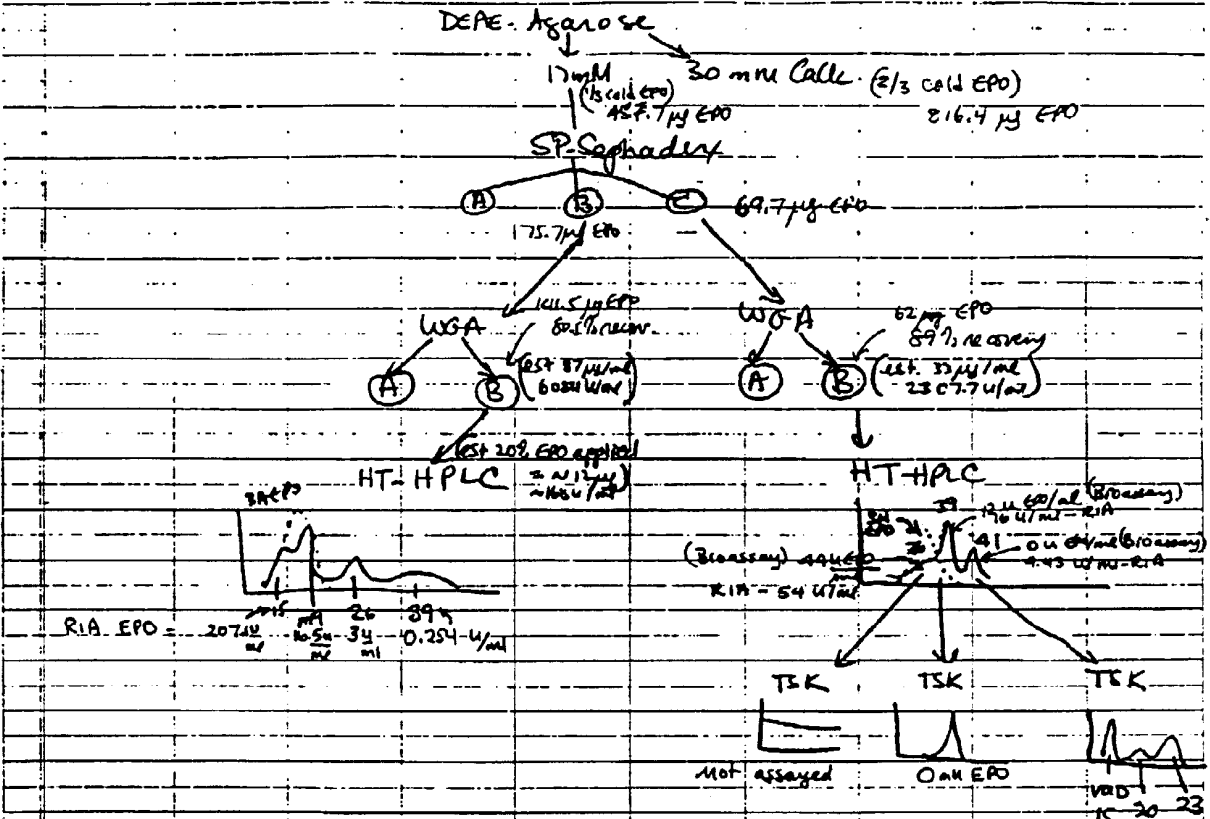
41

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Samples for RIA 5/23-5/24/84

① Samples from the urinary purification Lot 82

— = RIA calculation
 — = Bioassay (in vitro) calculation



② Serum-free roller bottle EPO production at 100 nM MTK amplification (cont.)
 Time Course exp
 Assay 24 hours, 69 hrs, 124 hrs, 147 hrs, 167 hrs, 207 hrs
 100% viable (1) (2) (3) (4) (5) (6) (7) (8) (9) (10) days
 100% viable 70% viable 75% viable 85% viable 90% viable 78% viable
 Rosem 2, 4, 8 + 9 days for a possible later assay.
 Use as a guideline for estimation of almost 50% for serum-free 100 nM MTK 65 hrs

③ Yield production human EPO (Syn. gene)
 Samples R. S. Elliott given to us on 5/22/84
 Super 151 U/ml control } Supernatant & cells
 + EPO }
 Cells pH 4.1 control } cells broken in glass beads
 + EPO }

FRISCH v. LIN
 INTER NOS 102,096
 102,097, 102,334
 LIN DOC NO L01149

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 Book No. _____ TITLE _____

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- (4) Kirin Patient samples
- (a) B-22 - Drive sera - repeat of last Kirin assay which showed this sample to be off scale - 44 u/ml
 - (b) B-24 - Repeat of last Kirin assay which showed this sample to be off scale - 13.7 u/ml - off scale 2 last assay
 - (c) 67 urine - LOT (1) 2.73 u/ml
 14 u/ml
 LOT (2) last assay = 1.81 u/ml
 LOT (3) 2.18 u/ml
 - (d) B-10 - LOT (1) last assay = 0.206 u/ml
 14 u/ml
 LOT (2) 0.137 u/ml
 LOT (3) 0.113 u/ml
 - (e) Kirin - normal sera - dose response was very abnormal in last Kirin assay - Repeat to see if it was due to a technical error - 40 u/ml
- (5) Ralph - h EPO (Syn gene) - CHO cell sup - 3 day CCM - OMK - 5.7 u/ml
 2 cell lines going - H3 - 3 1/2 day CCM - 18.2 u/ml
 Arrived 5/23/04
 Bill 5 1/2 day CCM - 18.2 u/ml

Protocol - 5x Trasyol
 Std EPO - SK #3 / PBS 10% TSA / Sample -> 4301 total vol.
 + Ab: 1601 1.5000 diln. 80 704 @ 2009 TB2
 Incubate 2 hrs at 37
 Cool on ice
 Add 125 EPO, 51044 Ampicillin
 Incubate o/n at 41° Room 6PM -> 9:30 AM
 Collect a 300 ul each into
 Note: higher conc "T-EPO added (along 0 more ab) since the
 70 pptn. of this 125 EPO had 1.502 to 33.2

FRITSCH v. LIN
 INTER NDS 102,096
 102,097, 102,334
 LIN DOC NO 101150

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AM 17 009837
 CONFIDENTIAL
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Sample	λ Pbs + 0.19.65A	gram	%total	%inhib.	EPO	EPO/ml		
1 0		430	5296	5273 100%	0	ED 0 = 11.76		
2 0		430	5251					
3 1.4u	9.5 λ D	425	5154	98.3	1.7			
4 2.5ml	12.5 λ D	420	4698	88.9	11.1			
5 5 ml	25 λ C	430	4314	81.4	18.6			
6 10	5 λ C	425	3008	56.2	43.8			
7 15	7.5 λ C	425	2710	40.7	59.3			
8 20	10 λ C	420	1602	29	71.0			
9 30	15 λ C	415	1039	18.1	81.9			
10 40	20 λ C	410	762	13.1	86.9			
11 50	25 λ C	405	704	11.6	88.4			
12 100	5 λ B	405	394	5.6	94.4			
13 200	10 λ B	420	257	2.9	97.1			
14 PT		430	111	105				
15 PT		430	100					
16 WG-A (B) - B	1:1000	50 λ	380 λ	336	4.5	95.5	140	2800
17 est. 87 μ g = 6084 μ /ml		20 λ	410 λ	426	62	93.8	80	1600
18 ml		10 λ	420 λ	521	8.0	92	70	7000
19		5 λ	425 λ	850	15	85	36	7200
20	1:10,000	20 λ	410 λ	2326	43	57	14	7000
21		5 λ	425 λ	4776	90.4	9.5	2.3	4000
22 WG-A (B) - B	1:1000	100 λ	330 λ	438	6.4	93.6	35	350
23 est. 33 μ g = 23077 μ /ml		30 λ	400 λ	508	7.8	92.2	70	2333
24 ml		15 λ	415 λ	816	13.7	86.3	39	2600
25		5 λ	425 λ	2718	10.5	49.5	12	2400
26		2 λ	428 λ	4418	83.4	16.6	42	2100
27 HT column - shoulder	1:100	300 λ	130 λ	337	4.5	95.5	110	36.7 μ /ml
28 of SP (B) WG-A (B)		100 λ	280 λ	183	7.7	93.3	81	54
29 est. biomass 3 λ = 200 μ l		25 λ	352 λ	720	12.9	87.1	41	54.7
30		37.5 λ	392.5 λ	1122	19.7	80.3	27	72
31		15 λ	415 λ	962	68.0	32.0	8	53.3
32		5 λ	425 λ	5170	98	2.0	1	
33 HT column - fraction # 29		10 λ	420 λ	366	5.1	94.9	105	10.5
34 of SP (B) WG-A (B)		5 λ	425 λ	414	6	94	90	18.0
35 est. biomass - at less than 100 μ l		130 λ	753	12.5	87.5	43	14.3	16.2 μ /ml
36		280 λ	972	16.8	83.2	52	21.3	
37 HT column - fraction # 41		10 λ	420 λ	758	12.6	87.4	42.9	4.29
38 est. biomass # 23 = 0		5 λ	425 λ	1534	27.7	72.3	21	4.2
39		130 λ	2296	42.4	57.6	41.4	4.8	44.3 μ /ml
40 HT column - fraction # 41	1:100	200 λ	230 λ	269	3.2	96.8	200	100
41 of SP (B) WG-A (B)		100 λ	330 λ	296	3.7	96.2	140	140
42 est. biomass has 20% of 200 μ l		20 λ	380 λ	462	6.9	93.1	80	160
43		20 λ	410 λ	710	12.9	87.1	41.4	20.7
44		7 λ	423 λ	2020	37.1	62.9	6.4	234.3
45		2 λ	428 λ	4543	85.9	14.1	3.6	18.0
46 HT column - fraction # 19	1:100	350 λ	80 λ	644	10.4	89.6	52	14.9
47 of SP (B) WG-A (B)		200 λ	230 λ	1008	17.5	82.5	32	16.0
48		100 λ	370 λ	1890	34.5	65.5	17.5	17.5
49		50 λ	380 λ	3214	60.7	29.3	8.3	17.6
50		20 λ	410 λ	5026	95.2	4.8	1.6	8.0
51		7 λ	423 λ	5218	100	-	-	-
52 HT column - fraction # 20		200 λ	230 λ	255	2.9	97.1	200	1
53 of SP (B) WG-A (B)		70 λ	460 λ	289	37	96.3	NO	2
54		20 λ	410 λ	386	4.3	90.7	60	3 - 3 μ /ml

FRITTSCH v. LIN 102,097, 102,334
INFER NOS 102,096 LIN DOC NO 101151

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AM 17 009838
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	Drawment ref.	Pool	λ PAST 0.17-0.50	Cap. m	% water	% T. m. d. b.	Exp.	Exp./ml
55	HT column	200λ	230λ	677	11.1	88.9	50	0.25
56	gSP (WGA) #29	70λ	360λ	1846	34.1	65.9	17.6	0.251 } 0.257+4ml
57		20λ	410λ	4284	80.3	19.7	5.2	0.26
58	JSK-SPO WGA (B) #24	200λ	230λ	5594	10.6	-	-	-
59	HT #29 (8 peaks)	100λ	330λ	5466	103.7	-	-	-
60		30λ	400λ	5362	10.6	-	-	0 EPO
61		10λ	420λ	5260	99.7	0.3	-	-
62		5λ	425λ	5498	104	-	-	-
63	JSK SPO WGA (B) #16	300λ	130λ	6290	119	-	-	0 EPO
64	HT #41 (probably 101)	200λ	230λ	5879	112	-	-	0 EPO
65	3 peaks	421	378λ	80λ	115	-	-	-
66		200λ	230λ	5945	113	-	-	0 EPO
67	60% max	200λ	230λ	5500	104	-	-	-
68		100λ	330λ	5519	105	-	-	0 EPO
69		30λ	400λ	5382	102	-	-	-
70		0λ	420λ	4929	93.3	6.7	-	-
71	JSK SPO WGA (B) #	20 bal						
72	HT #6	22 100λ						
73		24 100λ						
74		26 100λ						
75		27 100λ						
76		28 100λ						
77	HTO M-CO-6 days	79 100λ	1000 300 (30)	792	13.2	86.8	40	13.3
78	Cum. free rad. 100λ	30 100λ	100 330λ	1000	17.3	82.7	31	31
79	100 n M MTX	31 100λ	30 400λ	1513	272	72.8	21	70
80	[1.5 hrs]	32 100λ	10 420λ	2468	45.7	54.3	15	150.7 } 158.5
81		0 100λ	3 420λ	4211	79.4	20.6	53	177.3
82	HTO H-ETO 5 1/2 days #3	110 100λ	330λ	624	10.2	89.8	58	5.67
83		30λ	400λ	2263	41.7	58.3	15	5.0 } 5.7 ul ml
84		1200 100λ	300λ	4155	78.4	21.6	5.9	5.9
85		30λ	400λ	4883	92.4	7.6	1.9	6.3
86		0λ	420λ	5385	100	-	-	-
87	HTO H-ETO 5 1/2 days B11	110 100λ	330λ	362	5.0	95	110	11
88		30λ	400λ	648	10.5	89.5	53	17.7 } 18.2 ul ml
89		1100 100λ	330λ	1644	28.8	70.2	19	19
90		30λ	400λ	4278	79.8	20.2	5.4	18.0
91		0λ	420λ	5161	97.8	2.2	-	-
92	M-CO-Secum free rad. 100λ	Day 0 100 300	130λ	710	11.7	88.3	47	-
93	100 n M MTX (2 hrs)	1 An	330λ	957	6.4	93.6	31	-
94		1100 300	130λ	1213	21.4	78.6	25	-
95		1 An	330λ	1722	31.3	68.7	19	19 } 25 ul ml
96		30	400λ	3275	61.3	38.7	43	31
97	[1.5 hrs] Day 0 100 300	130λ	944	16.2	83.8	32	10.7	-
98		100	330λ	1162	20.4	79.6	27	27
99		30	400λ	1823	33.2	66.8	19	63.3 } 70.2 ul ml
100		10	420λ	3542	66.5	33.5	8.2	82
101		3	420λ	4706	89.0	11	2.5	93.3
102	[1.5 hrs] Day 0 100 300	130λ	554	14.5	85.5	37	-	-
103		100	330λ	1106	19.4	80.6	28	-
104		30	400λ	1654	30.7	69.3	19	63.3
105		10	420λ	2920	54.5	45.5	11	110 } 138.4
106		30	420λ	4328	81.7	18.3	5	160.7

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			λ PRS+ 0.1X SEA	c-p.m	% control	% inhibited	7p ⁰	7p ⁰ /ml	
107	167 Day 7	1:100 - 100λ	330λ	1066	18.6	81.4	30	30.0	
108		↓	30λ	400λ	1695	30.8	19	63.3	} 80.0 u/ml
109		↓	10λ	420λ	3125	58.4	10	100	
110		1:1000 - 30λ	400λ	4809	91.0	9.0	23	76.7	
111		↓	10λ	420λ	5584	105	-	-	
112	168 Day 10	1:100 - 100λ	330λ	1073	18.7	81.3	29	29	
113		↓	30λ	400λ	1635	29.6	20	66.7	} 77.8
114		↓	10λ	420λ	2855	55.1	11	110	
115		1:1000 - 30λ	400λ	4944	93.6	6.4	17	56.7	
116		↓	10λ	420λ	5553	105	-	-	
117	Sev Super pGT-191		100λ	320λ	5790	110	-	-	
118	SE 2-7 St2		30λ	400λ	5652	107	-	-	0 EPO in control
119	Yeast NeoR control		10λ	420λ	5529	104	-	-	
120		1:10	30λ	400λ	5624	107	-	-	
121		↓	10λ	420λ	5772	109	-	-	
122	Sev Super pGT-191 (EPO)		100λ	330λ	687	11.3	50	0.5	
123	SE 2-7 St2		30λ	400λ	918	15.7	34	1.1	
124	Yeast human EPO		10λ	420λ	1022	33.2	18	1.8	} 2.0 u/ml
125	OD of cells = 2.5	1:10	30λ	400λ	3248	60.8	7.5	2.5	
126		↓	10λ	420λ	4978	94.3	1.7	1.7	
127	Sev Cells pGT-191		100λ	320λ	5464	104	-	-	
128	SE 2-7 St2		30λ	400λ	5573	106	-	-	0 EPO in control
129	Yeast cells control		10λ	420λ	5490	104	-	-	
130		1:10	30λ	400λ	5353	101	-	-	
131		↓	10λ	420λ	5490	104	-	-	
132	Sev Cells pGT-191 (EPO)		100λ	330λ	1736	21.9	35	0.25	} 0.25 u/ml
133	SE 2-7 St2		30λ	400λ	3213	60.1	9.6	3.2	
134	Yeast human EPO		10λ	420λ	492	93.2	1.8	1.8	
135		1:10	30λ	400λ	5476	104	-	-	
136		↓	10λ	420λ	5574	106	-	-	
137	B-22 (DEWA)	1:100	50λ	380λ	1406	25.2	74.8	22	44
138	NEG		25λ	405λ	2694	50.1	49.9	11	44
139			12.5λ	4125λ	4158	78.4	21.6	57	46.6
140		↓	6.25λ	423.75λ	4986	94.6	5.4	0.7	-
141	B-24	original	50λ	420λ	538	8.4	91.6	67	13.4
142	NEG		2.5λ	422.5λ	885	15.1	84.9	35	14
143		1:10	50λ	480λ	633	10.2	89.8	53	10.6
144		↓	25λ	405λ	1115	19.5	80.5	28	11.2
145	B-7 (normal diluted)		100λ	320λ	304	3.8	96.2	140	1.4
146	(1:4 diluted)		50λ	380λ	440	6.5	93.5	35	1.7
147	NEG		25λ	405λ	655	10.6	89.4	52	2.08
148			12.5λ	412.5λ	1191	21	79	26	2.08
149	B-7 (normal diluted)		100λ	320λ	306	3.9	96.1	140	1.4
150	(1:4 diluted)		50λ	380λ	453	6.7	93.3	32	1.64
151	NEG		25λ	405λ	891	15.2	84.8	34	1.36
152			12.5λ	412.5λ	1542	27.8	72.2	21	1.68
153	B-10 (normal diluted)		300λ	130λ	1030	17.9	82.1	30	0.1
154	(1:4 diluted)		200λ	230λ	1626	30	70	20	0.98
155	NEG		100λ	310λ	3274	61.3	38.7	9.3	0.93
156			50λ	380λ	4835	91.5	8.5	2.2	0.1374 u/ml
157	B-10 (normal diluted)		300λ	130λ	1201	21.2	78.8	35	0.83
158	(1:4 diluted)		200λ	230λ	1947	35.6	64.4	17.5	0.875
159	NEG		100λ	320λ	3734	70.2	29.8	7.5	0.75
160			50λ	380λ	4921	93.2	6.8	0.8	0.115 u/ml

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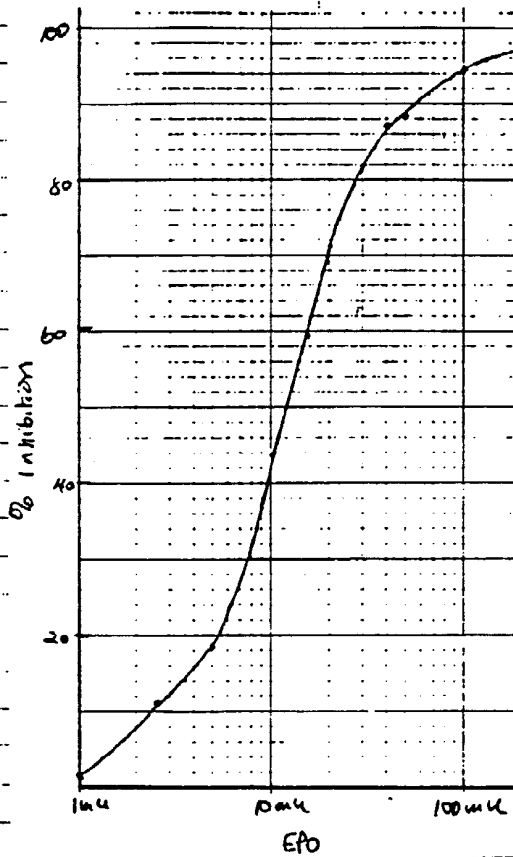
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			λ ^{0.1% 0.1% 0.1%}	cpm	% control	% inhib	EPO	EPO/ml	
167	Keuro	300 λ	130 λ	3435	45.1	54.9	13.5	45 μ /ml	Actual
168		200 λ	220 λ	3907	73.6	26.4	7.0	35 μ /ml	
169	(Davidson)	100 λ	320 λ	5706	108.4	-			
170	NEA								



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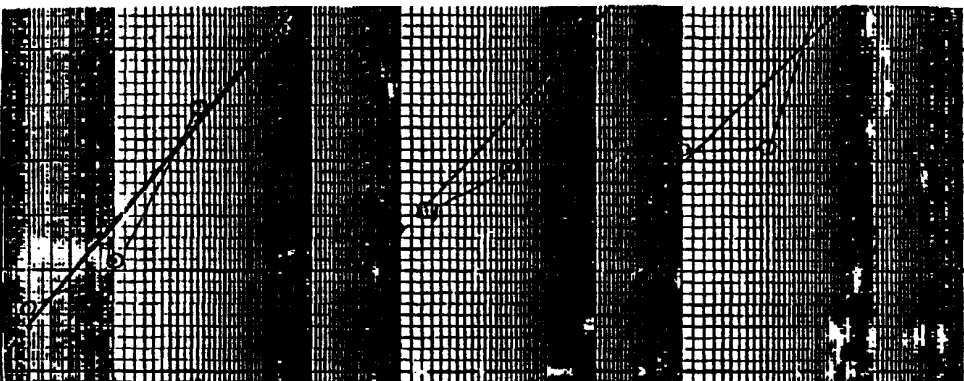
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Project No. _____ CMO - the EPO
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(Hypoxic) 11-4-84 - 4-8-84 JAZ

VE-37 (OPN)	Date M/F/yr	% SE	U	Human Urinary Erythropoietin Secondary Standard	b ± s _b	S _{pr}	n	r
	1:2 4.42 ± 0.201	42.7	6.10.8		30.8 ± 3.2	6.6	25	0.89
	1:4 4.42 ± 0.201	31.7	21.60.4					
	1:8 2.12 ± 0.201	24.8	17.0.2					
	1:16 0.52 ± 0.201	19.9	14.0.2					
		8.7	14.0.2					
H3-1	0.2 2 ± 0.201	39.8	42.0.7	UT- hem 22.4	25.6 ± 7.0	10.5	19	0.66
H3-2	0.1 2 ± 0.201	22.0	24.0.64	5.6 ± 3.9				
H3-3	0.17 2 ± 0.201	21.0	24.0.17	8.4				
H3-4	0.017 2 ± 0.201	20.8	14.1.0.17	6.1				
H11-1	0.1 2 ± 0.201	42.3	46.0.64	8.4	272 ± 4.6	9.8	25	0.76
H11-2	0.05 2 ± 0.201	47.7	40.0.1.25	15.0				
H11-3	0.012 2 ± 0.201	20.0	11.0.8.2	20.8				
H11-4	0.0012 2 ± 0.201	19.8	14.0.0.15	15.0				
H11-5	0.0002 2 ± 0.201	18.8	15.1.0.11	12.6				



0.05 0.1 0.2 0.4 0.8 Erythropoietin (U) VE-37 0-0

H3 & H11 are CMO CCM, 3'h r² h day respectively. F... clones producing the EPO

R1A in vitro in vivo
 H3 sb3-57 (20) - 105 ± 2.1 44 - 5.6 ± 4.3
 B1 sb3-122 (20) - 224 ± 1.06 44 - 16.3 ± 3.0

FOR ALL THE CMO MATERIAL, AS FOR HUMAN CMO, ALL EPO ACTIVITY IS MONITORED IN VIVO!!

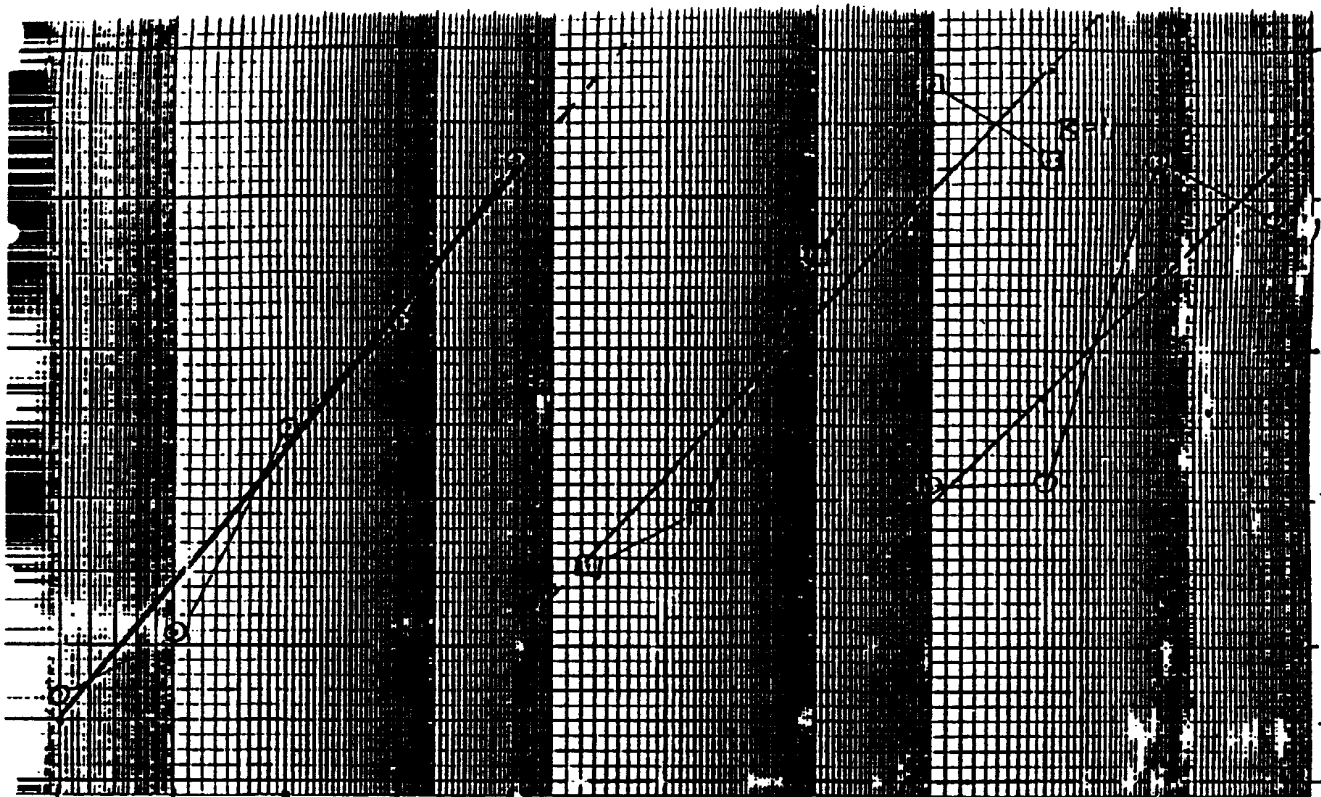
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.05 0.1 0.2 0.4 0.8 Enthropoietin (U) UE-37 6-0

H3 rB11 are CHO CCM, 3' h + 5' h days respectively. f. 2 clones producing the EPO

RIA	n vho	n vvd
563-57 70-55552	(*)-105502.1	64-56543
563-122 70-72522	(*)-22495106	64-16853.0

FOR ALL NEW CHO MATERIAL, AS FOR MONKEY CHO, ALL EPO ACTIVITY IS MAINTAINED IN VIUD!!
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H3 + B11 are CHO CCM, 3 1/2 + 5 1/2 days respectively. F. 2 clones producing the EPO

RIA	in vitro	in vivo
543-57 70-55532	(#2)-105962.1	64-56543
543-10.2 73-102522	(#2)-22495106	64-16853.0

FOR HUMAN CHO MATERIAL, AS FOR MONKEY CHO, ALL EPO ACTIVITY IS MAINTAINED IN VIVO!!
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7/3/84 EPO 2114

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7/3/84 RIA for EPO

Sampled

- ① Amgen/Kirin Purc EPO - Adj. 10 u/ml
- ② CHO - hEPO - #3, 0.1
- ③ A Dukes UE-35
- ④ Goldwasser U-4-83 pure EPO
- ⑤ CHO - mEPO - line 4.1 - Bio response
- ⑥ CHO - hEPO - 2 lgc stock plus

- ⑦ E. coli in EPO - 100 mg/ml - 100 u/ml - WFA → DEAG
- ⑧ I. Strickland - mEPO serum - final 1g/500 u/ml
- ⑨ Elliott - yeast - hEPO 6-29-84
- ⑩ mouse urine - CHO-EPO pure injected serum A. 6/29 RIA

① Amgen/Kirin Purc EPO - Using protein conc. estimate by 17% yield, adjust conc. of EPO to 10 u/ml. Dilute this stock 1:10 in PBS + 0.1% BSA & assay in RIA. 5 m lgc assay. Repeat R. 1/20.4 + RIA cal. Since some of the higher values gave poor dose response. 41 of 175 u/ml = 4.89 u/ml BSA → 10 u/ml based on protein. 10.67 u/ml = 10 u/ml stock.

② 75.90% ETOH ppt of Amgen/Kirin lot for urine purification. 1.5970 diln → 10 u/ml estimate based on 24-50% recovery to that step. Use at 1:10 diln → 14 u/ml working stock. Repeat from last assay to at the 10 u/ml (instead of 10 u/ml level). 14.4% + 0.9 u/ml = 10 u/ml stock.

③ hEPO - #3 - 3/6 d. 0.1 u/ml NIX 5/23/84. Purc RIA = 5/23/84 = 5.7 u/ml. 5.55 u/ml.

CHO hEPO - #4 - 5/12 d. 0.1 u/ml NIX 5/23/84. Purc RIA = 5/23/84 = 17.83 u/ml. 17.83 u/ml.

④ P. Dukes UE-35. Adjust to 100 u/ml based on label in vivo titer (12 u/ml). Make a 1:100 diln → 1 u/ml for assay in RIA. P. Dukes UE-35 is used as the in vitro bioassay standard. It tends to assay as 1/2 the stated in vitro units in Bethesda-Clement's RIA (Garcia also) but helps determine if same applies to our system since we are able to see units per ml one sample when assayed in in vitro + RIA systems. 1213.01 u/ml for 1 u/ml diln.

⑤ E. Goldwasser EPO from plain. 12.9.83 HT prep. Was anticipated that we could use this EPO as a standard for all our assays. However, in vitro - in vivo activities are only 40.50% anticipated amt. of units. Check by RIA. 1.33 ± 0.15 u/ml for 1 u/ml diln.

⑥ CHO - mEPO - line 4.1. 3 d. 0.1 u/ml + serum - 100 u/ml NIX 7-2-84. Sample cells for bio response. Check to ensure cells still producing EPO. 3/6 u/ml.

⑦ CHO - hEPO - 2 lgc pools of same in the lab. a) largest pool of material → 100 - 100 u/ml total - all different times of collection at diff. [mix] of 300 u/ml collected = starting in May... 53.4 u/ml.

b) 40 u/ml total - 20 u/ml - 10 d. CCM 7 ~ 0 u/ml NIX. Collected, starting in May. 20 u/ml - 10 d. CCM. 70.1 u/ml.

⑧ Human EPO - coli - 10% lactate - 100 mg/ml pellet → 15K. Check # 19. No titer - why?

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19844 Tom Strickland purification samples		
Large USA prep. of SE: MEPO. CCM - CPO		
Use 14M ¹⁰⁰ today. old sample having a filter of 212 u/ml		
column load USA - estimated filter 300 u/ml - necessary - 333 u/ml		
low flow samples - fractions	#10 - est 0-150 u/ml	18 u/ml
	#20 - est 75-200 u/ml	130 u/ml
	#50 - est 150-225 u/ml	260-500 * u/ml
	#80 - est 150-225 u/ml	260-925 * u/ml
to check for column overloading - amt of 315 spiked EPO ↑ after fraction #20.		
USA - Eluent - est. 3000-6000 u/ml		3800 u/ml
↓ dialysis → 2x ↑ vol.		
EAE Agarose load - est. 2700-5500 u/ml		2250 u/ml
Elute E 30 mM CaCl ₂ - est. 1100 u/ml * All 315 head!		1597 u/ml
Elute E 100 mM CaCl ₂ - est. 550 u/ml		19.3 u/ml

least - 4x EPO samples R. Steve Elliott - 6-29-84 A600 = 7.9
 New yeast construction for a factor secretion system where hypO replaced (see 2)
 by 6-29-84 frequency of deletion by the EPO unit.
 Control 1 - EPO samples - each concentrated 170x
 YSDP4 - PVE SE EPO - Sup Conc 170x
 YSDP4 - Control - Sup " "

Treat both control + the EPO + ENDO H
 Endo H treated samples run on 2.5% SDS gel + Coomassie stained. See 4 new
 bands in EPO samples that are not present in controls. These bands are same as
 those seen in prior construction, as Ag stained gel. 4 bands together est ~ 4.5 μg prot
 YSDP4 + PVE SE EPO - Sup - ENDO H - 0.50 u/ml? Don't require not 11 tests
 " Control - Sup - ENDO H - 0 u/ml
 YSDP4 + PVE SE EPO - Sup - ENDO H - 361.25 u/ml " " " " *
 " Control - Sup - ENDO H - 0 u/ml

Endo H treated 5 min || than the non-endo H treated. Sample of old culture 2 years earlier superimposed
 ... Review of mouse urine from the experimental group receiving CPO cells + EPO. Some
 in last assay 6/2/84. These animals are negative, suggesting either that the titer
 of EPO was much lower than expected, or that the EPO had been degraded sometime
 during the collection + dialysis steps.

Date	Day Post inj	Total Volume Yd	Neutrophil	
4/17	5	41 ml	50	NO TITER EVEN AT THE
4/18	6	37 ml	N)	HIGHER ALIQUOT SPES.
4/20	8	0.95 ml	66.1	
4/24	12	0.95 ml	704 (D.U)	

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ED₅₀ = 22 mU

10) = 20008 cpm 61.4/100

SAMPLES	100% AMGEN EPO	100% PAS+ 0.1% BSA	CPM	% Control	% Inhibit	EPO	EPO/ML
STDS	445	4864					
0	445	1638 *		100%	0		
1mU = 52D	440	5464		96.8	3.2		
25mU = 252D	430	5284		92.9	7.1		
5mU = 252C	440	5158		91.1	8.9		
10mU = 52C	440	4978		87.7	12.3		
15mU = 752C	435	3926		68.1	31.9		
20mU = 102C	435	2181		54.3	45.7		
30mU = 152C	435	2182		35.7	64.3		
40mU = 202C	425	1665		26.1	73.9		
50mU = 252C	420	1384		28.9	75.1		
100mU = 52B	440	782		9.7	80.3		
200mU = 102B	435	575		5.8	74.2		
PI	495	257		26.3			
PI	435	268					
100% AMGEN/KLIN Pure EPO 100% 100	395	1153	16.6	89.4	58	9.67	
1u/ml sample adjusted for protein concentration data 40	405	1652	25.8	74.2	40	10.0	5.8
20	415	1978	31.9	68.1	32	10.67	10.67 u/ml
10	425	2378	42.7	57.3	22	11.0	12.25 u/ml
5	435	4428	72.5	22.5	12	12	130.46 u/ml
150% ETOH ppt - lot 92 u/ml 100	395	932	10.6	89.4	91	15.17	1.9
100% of 10 u/ml stock 40	405	1164	16.8	83.2	82	24.0	14.83 u/ml
20	425	2558	42.7	57.3	265	13.25	
10	435	3884	67.4	32.6	155	15.5	
100% ETOH 100	295	918	13.1	86.9	74	4.53	
H3 - 3 1/2 ccM stock 100	345	1239	12	81.8	51	5.1	5.5
75	370	1474	22.5	77.5	43	5.73	5.67 u/ml
50	395	1978	31.9	68.1	32	6.4	
25	420	3595	69.4	30.6	14	5.6	
BH - 5 fold conc chaly 100	385	898	11.8	83.2	82	13.67	3.25
100% H3 EPO 40	405	1068	15	85	64	16.0	
20	425	172	27.1	72.9	37	18.5	17.83 u/ml
10	435	3354	57.5	42.5	19	19	
5	440	4632	82.2	17.8	11	22	
P. Dukes UE:35 adjust 100	345	620	7.6	92.4	130	1.3	2.1
to 1u/ml 75	370	841.054	89.108	89.2	88	1.17	1.21 u/ml
stock = 10 u/ml 100	395	1168	16.8	83.2	57	1.14	
25	420	2516	41.9	58.1	27	1.08	
10	435	4178	72.8	27.2	13.5	1.35	
E. Gullwasser Pure EPO 100 100	365	856	11	89	87	1.09	1.05
12-19-93 HT 1u/ml 40	405	1112	15.8	84.2	60	1.5	1.33 u/ml
20	425	2550	42.5	57.5	27	1.35	
10	435	3950	69.3	30.7	14	1.4	
M: EPO - clone 9.1 3d ccm + 100 100	345	1433	21.8	78.2	45	4.6	4.6
secum (for Bronx) 40	395	1572	24.4	75.6	40	"	"
7-2-84 - 100M HTK 20	420	1726	29.1	70.9	36	"	"
25	430	2323	39.3	60.7	28	224	
6	434	3328	57	43	19	316	316 u/ml
EPO - Big Pool of ccm - 100 100	345	584	5.7	94.3	200		
100% Acadat H3 100	395	584	6	94	209		
1 Def. HTX 0 - 200 100	420	878	22	92.3	190	52	52.4 u/ml
2 Def. HTX 0 - 200 100	430	1030	14.3	85.7	66	52.3	

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SAMPLE	λ PBS r 0.1% BSA	cpm	% Control	% Inhibitor	EPO	EPO/ml	
Ab-HuEPO - By zone at com 20 ml 4.5 com	1:1000	345	87	5.7	94.3	200	too high
	50	345	552	5.4	94.6	200	too high
	25	420	628	6.8	93.2	160	64
	12.5	430	872	11.3	88.7	85	68
	6.25	434	1028	21.2	78.8	47	78.3
Ecoli-HuEPO - 19 kune G Rach on ISK - Rach (P) Wash	1:1000	345	5403	95.6	4.4	-	-
	20	415	5457	96.6	3.4	-	710 EPO hbr
	10	435	4888	86	14	-	-
	1:1000	415	5442	96.4	3.6	-	-
	1	435	5592	99.1	0.9	-	-
H. PPO - TS Purif WFA col 6/26/04 Col. Load	1:1000	245	2096	34.1	65.9	31	-
	100	345	2868	48.5	51.5	22.5	225
	50	345	3570	60.4	39.6	18	310
	25	420	4551	79.8	20.2	11.5	460
	12.5	430	5220	92.2	7.8	3.6	288
Wash of column # 10	1:1000	345	1109	15.7	84.3	60 Tpm	18 u/ml
	1:100	345	1630	25.4	74.6	40 Tpm	-
	1:1000	345	3546	61.1	38.9	18	-
	1:100	345	585	11.6	88.4	too hi	-
Wash of column # 30	1:1000	345	1224	17.9	82.1	too hi	-
	1:100	345	1756	27.8	72.2	too hi	-
	1:1000	345	8215	73.6	26.4	13	130 u/ml
	1:100	345	1024	11.2	88.8	too hi	-
Wash of column # 50	1:1000	345	1479	22.6	77.4	too hi	-
	1:100	345	2569	42.9	57.1	26	260
	1:1000	425	4960	87.4	12.6	10	500
	1:100	345	1016	14	86	too hi	-
Wash of column # 100	1:1000	345	1446	22	78	too hi	-
	1:1000	345	2594	43.4	56.6	26	260
	1:100	425	4698	82.5	17.5	10.5	525
	1:1000	345	1984	32	68	too hi	-
WFA eluant	1:1000	245	2180	35.7	64.3	too hi	-
	100	345	1984	32	68	too hi	-
	50	345	2588	61.9	38.1	17	3400
	25	420	4634	81.3	18.7	10.5	4200
	12.5	430	4904	86.3	13.7	-	-
DEAE Agarose of WFA Column Load	1:1000	245	2232	36.6	63.4	too high	-
	100	345	2955	58.8	41.2	21	2100
	50	345	4326	75.6	24.4	12	2400
	25	420	4718	82.9	17.1	10.5	-
	12.5	430	5405	95.7	4.3	-	-
30 mM DEAE-Agarose	1:1000	345	1806	28.7	71.3	too hi	-
	10	345	2247	36.5	63.1	"	-
	25	420	2824	48.6	51.4	22	880
	12.5	430	3611	62.3	37.7	17	1360
	6.25	434	4733	83.2	16.8	11	1833
100 mM DEAE-Agarose	1:1000	245	2610	43.7	56.3	too hi	-
	10	345	3854	66.8	33.2	16	16
	50	345	4634	81.3	18.7	11	22
	25	420	5158	91.1	8.9	5	20
	12.5	430	4470	78.3	21.7	-	-
Yeast 6-28-R4 EPO Sup Yeast - 170 + conc YSDP 4 + P46 S.C.R.P.	1:1000	345	527	4.9	95.1	too hi	-
	100	415	576	5.8	94.2	200	666.7
	10	435	814	10.3	89.7	92	920
	1:1000	30	415	1370	20.7	79.3	48
	10	435	2137	34.9	65.1	31	3100

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SPDF 150
 main 5 5000
 COSU/ml
 72.2 u/ml
 170 x conc =
 4248 u/ml
 JRCMC

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SAMPLES		APPM	cpm	% Control	% Inhibitor	EPO	EPO / ml	
	10000	20)	415	3670	63.4	36.5	16.5	5500
		10)	435	4988	87.7	12.1	10	10,000
		3)	440	4806	84.5	15.5	-	
Yeast - 6-28-84 Control Sup	1000	ml	345	5248	92.7	7.3	3.0	CONTROL IS NEGATIVE
170x conc		2)	415	5572	99.1	0.9	-	
YSDP4 - Sup		ml	435	5446	96.4	3.6	-	
Yeast 6-28-84 FENDH that Sup	1000	ml	345	772	8.5	91.5	120	120
YSDP4 + PVG serpo		2)	415	988	12.1	86.9	72	240
170x conc		ml	435	2138	34.9	65.1	30.5	305
		2)	415	4476	78.4	21.6	12	400
		ml	435	5161	91.1	8.9	5	500
	10000	30)	415	5446	96.4	3.6	-	
		ml	435	4419	77.3	22.7	-	
		3)	440	5034	85.8	14.2	-	
Yeast 6-28-84 Control	1000	ml	345	4728	82.1	16.9	10.5	-
Sup + 170x conc		20)	415	8740	64.7	35.3	17	CONTROL IS NEGATIVE
YSDP4 - Sup		ml	435	5704	101	-	-	
1000 - 1000 - 1000 - 1000	200	ml	245	5564	98.6	1.4	-	
Day 5		ml	345	5359	94.8	5.2	-	
Day 6		200)	245	5177	91.4	8.6	45	
		ml	345	5430	96.1	3.9	-	No EPO measured -
Day 8		200)	245	4671	82.1	17.9	105	in mouse urine
		100)	345	4762	82.7	16.3	10.2	
		ml	345	5304	93.8	6.2	-	
Day 12		200)	245	5551	98.4	1.6	-	
		ml	345	4364	76.9	23.1	-	
		ml	395	5706	97.5	2.5	-	
		2)	420	5326	94.2	5.8	-	

Probes
 SA Transgel
 with sample + P65 + 0.1% BSA
 40x 1.500 diln SC204 3009 1B2
 Incubate 1 1/2 hrs 37°
 All blig 1/4 "EPO - Antigen reactivity of (ATF) EPO ml = 20008 cpm
 Incubate 0° from 6PM -> 10 AM
 Collect 0.3 ml Tech 100 as usual

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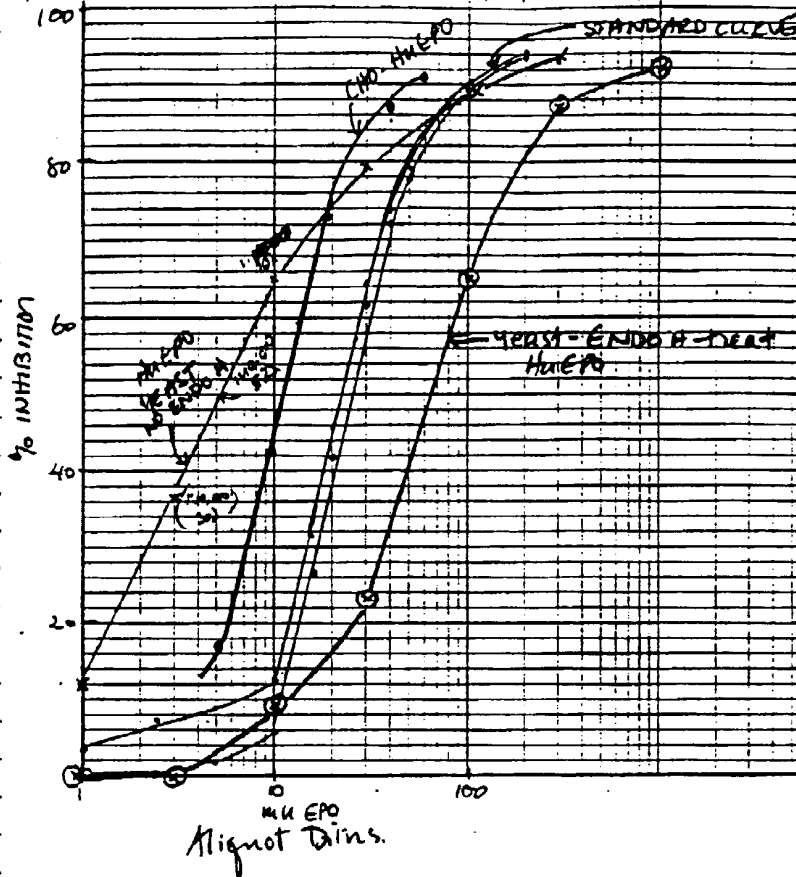
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