

V79 COLONY FORMING ASSAY

Experiment Name : $^3\text{HTdR}$ (100% labeling, cluster, suspension);
 Experiment performed by : A. Bishayee

Exp. # : 1;
 Date: 12/16/99

1. Set the rocker-roller at 37°C incubator with 5% CO_2 , set the Coulter Counter, wash cells (from two 150 cm^2 flask, subcultured 1:2, 24h before) with PBS, trypsinize cells, each resuspend in 9 ml MEMB, pool, pass five times through 3 cc syringe with 21 gauge needle, perform cell count by transferring 100 μl in Coulter cup containing 20 ml isotone (Coulter balanced electrolyte solution)
2. Dilute to $\sim 4,000,000$ cells/ml in MEMB [Actual count : 4,137,353 cells/ml]
3. Transfer 1 ml of cell suspension into 14 12 ml tubes (Falcon plastic test tube, 17×100 mm) labeled 1-14 both on cap and wall
4. Keep the tubes in the roller for 3-4 h at 37°C , 5% CO_2 Date/Time: 12/16/99; 3-00 pm.
5. Prepare MEMB containing radioactivity in hood
 $120\ \mu\text{l}\ ^3\text{HTdR}$ (Stock : $\mu\text{Ci}/\mu\text{l}$ on 10/20/99) + 4.88 ml MEMB
6. After 3-4 h, remove test tubes from roller and add MEMB with or without radioactivity according to Table below. Date/Time: 12/16/99; 7-15 pm.

Tube #	³ HTdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ³ HTdR (ml) [²⁴ uCi/m l]
1	0	1.0	1.0	0
2	0	1.0	1.0	0
3	0.5	1.0	0.96	0.04
4	2	1.0	0.834	0.166
5	4	1.0	0.67	0.33
6	8	1.0	0.34	0.66
7	10	1.0	0.16	0.83
8	0	1.0	1	0
9	0	1.0	1	0
10	0.5	1.0	0.96	0.04
11	2	1.0	0.834	0.166
12	4	1.0	0.67	0.33
13	8	1.0	0.34	0.66
10	10	1.0	0.16	0.83

7. Return test tubes to roller for 12 h . Date/Time: 12/16/99; 7-30 PM.
8. Next day, while test tubes are in roller label 10 gamma-tubes (13 X 100 mm VWR glass test tube)
9. After ~12 h incubation period, remove tubes and centrifuge at 2000 rpm at 4°C for 10 min (*precooled centrifuge*). Date/Time: 12/17/99; 9-15 A.M.
10. Remove buckets from centrifuge and carefully remove 150 µl of supernatant and place in prelabeled gamma-tube.
11. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
12. Centrifuge tubes for 10 min at 2000 rpm, 4°C
13. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C

17. Decant supernatant, click tubes, vortex
18. Resuspend for tube 8-14 in 2 ml of MEMA and transfer to 10.5°C for 72 h
19. For tube 1-7, transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
20. Again add 200 ul ice cold MEMA ~~with or without 10% DMSO~~, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
21. Centrifuge tubes for 5 min at 1000 rpm, 4°C
22. Transfer tubes at 10°C for 72 h. **Date/Time:** 12/17/99; 11-15 a.m.
23. Transfer 30 ul supernatant in three sets of 6 ml scintillation vials containing 6 ml liquid scintillation cocktail (Ecolume) from 150 ul supernatant removed earlier (Step 10) and count them for radioactivity **Date/Time:** 12/17/99; 1-00 pm.
24. After 72 h, for tubes 1-7, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to 8 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet **Date/Time:** 12/20/99; 9-00 a.m.
25. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes. For tubes 8-14, add 8 ml wash MEMA
26. Centrifuge all tubes for 10 min at 2000 rpm, 4°C (precooled centrifuge)
27. Labeling and preparation of dilution tubes and colony dishes
 - load 66, 60 mm petri dishes with 4 ml MEMA
 - load 40 sterile tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.
28. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
29. Centrifuge tubes for 10 min at 2000 rpm, 4°C
30. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
31. Centrifuge tubes for 10 min at 2000 rpm, 4°C
32. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
33. Determine cell concentration by transferring 100 µl to Coulter cup
34. Vortex tube, transfer 0.5 ml into dilution tube X.5, vortex tube X.5, transfer 0.5 ml into dilution tube X.4, vortex tube X.4 and transfer 0.5 ml to tube X.3, vortex tube X.3 and transfer 0.5 ml to tube X.2 and vortex. Keep tubes on ice.
35. Transfer 1 ml from dilution tubes into dishes labeled X.2, X.3, X.4 (in triplicate). Only X.2 should be seeded for control T-tubes.
36. Transfer 200 µl of cell suspension (in triplicate) to 20 ml scintillation vial containing 6 ml cocktail (AquaSol) Ecolume
37. Incubate petridishes for 1 week

38. Count vials for radioactivity

Date/Time : 12/20/99 12:00 noon

39. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol.

Stain colonies with 0.05% crystal violet

40. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

12/16/99

Initial count = 1020, 1035, 1048

Avg count = 1034

Cell conc = 4,137, 333 Cells/ml

30uL medium

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 FRI 17 DEC 1999 12:51
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SDR:N RS232:N
 #: 1 AGC:N BCF:N RCM:N
 CHANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
 DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 1.00000
 HALF LIFE(DAYS):N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	16.00	50.00	1.00	1.84	90.0	
2	**	2	18.00	47.14	1.00	3.87	93.0	
3	**	3	14.00	53.45	1.00	5.88	94.0	
4	**	4	13.00	55.47	1.00	8.01	91.0	
5	**	5	19.00	45.88	1.00	9.99	93.0	
6	**	6	17.00	48.51	1.00	12.02	93.0	
7	**	7	7356.00	2.33	1.00	14.05	93.0	
8	**	8	7692.00	2.28	1.00	16.13	93.0	
9	**	9	7238.00	2.35	1.00	18.31	91.0	
10	**	10	29741.18	1.99	0.34	19.57	94.0	
11	**	11	30829.33	1.86	0.38	21.01	92.0	
12	**	12	29457.54	1.93	0.36	22.39	93.0	
13	**	13	52386.66	1.84	0.23	23.68	92.0	
14	**	14	58972.09	1.78	0.22	25.17	93.0	
15	**	15	59765.00	1.83	0.20	26.34	93.0	
16	**	16	111514.29	1.43	0.17	27.60	93.0	
17	**	17	117389.38	1.74	0.11	28.53	96.0	
18	**	18	112108.57	1.43	0.17	29.79	92.0	
19	**	1	142579.83	1.54	0.12	31.31	91.0	
20	**	2	132304.00	1.56	0.12	32.31	90.0	
21	**	3	133668.70	1.35	0.16	33.51	91.0	
22	**	4	19.00	45.88	1.00	35.63	93.0	
23	**	5	13.00	55.47	1.00	37.62	91.0	
24	**	6	14.00	53.45	1.00	39.58	93.0	
25	**	7	13.00	55.47	1.00	41.57	91.0	
26	**	8	14.00	53.45	1.00	43.63	92.0	
27	**	9	13.00	55.47	1.00	45.71	92.0	
28	**	10	6990.00	2.39	1.00	47.83	92.0	
29	**	11	6690.00	2.45	1.00	49.87	91.0	
30	**	12	6731.00	2.44	1.00	51.90	90.0	
31	**	13	30522.86	1.94	0.35	53.42	92.0	
32	**	14	30834.29	1.93	0.35	54.94	93.0	
33	**	15	29960.00	1.95	0.35	56.26	94.0	
34	**	16	57888.37	1.79	0.22	57.49	91.0	
35	**	17	59434.29	1.96	0.17	58.73	93.0	
36	**	18	58883.33	1.94	0.18	60.34	94.0	
37	**	1	123766.87	1.41	0.16	61.59	92.0	
38	**	2	116726.66	1.51	0.15	62.72	93.0	
39	**	3	117717.79	1.44	0.16	63.91	94.0	
40	**	4	141011.44	1.27	0.17	65.17	90.0	
41	**	5	149037.23	1.19	0.19	66.50	92.0	
42	**	6	137217.39	1.45	0.14	67.57	92.0	

TABLE-1

Expt. # : 1

Date/Time : 12/17/99; 1-00 PM.

Tube #	Medium count for 30 ul (cpm)	Avg. cpm	dpm [cpm/ 0.65] 0.58	μ Ci/ml (A _c) on counting [dpm/66600]	μ Ci/ml (A _o) on addition [A _c /e ^{-λt}]
1					
2					
3		7428	12808	0.192	
4		30009	51739	0.777	
5		57041	98346	1.47	
6		113670	195983	2.94	
7		136183	234798	3.53	
8		6			
9					
10		6803	11730	0.176	

11	30438	52480	0.788
12	58735	101267	1.52
13	119403	205067	3.09
14	142421	245554	3.69

Roome cells

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 MON 20 DEC 1999 12:03
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
 H#: 1 ADC:N QCF:N RCM:N
 CHANNEL 1-LL: 0 UL: 400 ZSIGMA: 2.00 BKG SUB: 0.00 BKG ZSIB: 0.00 LSR: 0
 DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000
 HALF LIFE(DAYS):N

SAM	POS	CH	CPM	ZSIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	9.00	66.67	1.00	1.85	111.0	
2	**	2	3.00	115.5	1.00	3.93	113.0	
3	**	3	17.00	48.51	1.00	6.11	110.0	
4	**	4	9.00	66.67	1.00	8.08	108.0	
5	**	5	12.00	57.74	1.00	10.11	110.0	
6	**	6	15.00	51.64	1.00	12.13	109.0	
7	**	7	8059.00	2.23	1.00	14.10	112.0	
8	**	8	6422.00	2.50	1.00	16.53	110.0	
9	**	9	7362.00	2.33	1.00	18.52	110.0	
10	**	10	35835.71	2.00	0.28	20.22	110.0	
11	**	11	33800.00	1.94	0.31	21.56	110.0	
12	**	12	34533.33	1.96	0.30	22.82	111.0	
13	**	13	59855.00	1.83	0.20	24.20	109.0	
14	**	14	64148.57	1.89	0.17	25.44	111.0	
15	**	15	64057.14	1.89	0.17	26.68	111.0	
16	**	16	131226.98	1.37	0.16	27.88	109.0	
17	**	17	125973.33	1.45	0.15	29.02	111.0	
18	**	18	125870.00	1.78	0.10	30.30	110.0	
19	**	1	127423.31	1.39	0.16	31.55	109.0	
20	**	2	138673.92	1.45	0.14	32.62	110.0	
21	**	3	167006.12	1.21	0.16	33.82	112.0	
22	**	4	659.00	7.79	1.00	35.70	110.0	
23	**	5	326.00	11.08	1.00	37.82	110.0	
24	**	6	378.00	10.29	1.00	40.39	111.0	
25	**	7	82.00	22.09	1.00	42.42	110.0	
26	**	8	107.00	19.33	1.00	44.39	109.0	
27	**	9	46.00	29.49	1.00	46.27	112.0	
28	**	10	5954.00	2.59	1.00	48.51	112.0	
29	**	11	6044.00	2.57	1.00	50.43	111.0	
30	**	12	6042.00	2.57	1.00	52.46	110.0	
31	**	13	28235.62	1.97	0.36	53.83	110.0	
32	**	14	25105.00	2.00	0.40	55.20	111.0	
33	**	15	26925.00	1.93	0.40	56.77	110.0	
34	**	16	50400.00	1.88	0.23	58.37	111.0	
35	**	17	43392.46	1.87	0.26	59.66	110.0	
36	**	18	49087.50	1.84	0.24	60.82	109.0	
37	**	1	85456.52	1.84	0.14	61.92	110.0	
38	**	2	77659.58	1.66	0.19	63.23	109.0	
39	**	3	73485.51	1.76	0.14	64.30	113.0	
40	**	4	111049.08	1.49	0.16	65.49	107.0	
41	**	5	104693.33	1.60	0.15	66.62	106.0	
42	**	6	113936.17	1.37	0.19	67.94	110.0	

TABLE-2

Expt. # : 1

Date/Time : 12/20/99; 12-00 noon

Tube #	Radioactivity for 200 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.65] 0.58	μ Ci/ml (A _i) on counting [dpm/444000]	μ Ci/ml (A _o) after 12 h incubation [A _i e ^{-λt}]
1					
2					
3		7281	12553	0.0283	
4		34722	59866	0.1348	
5		62686	108080	0.2434	
6		12769 141401	220154 223795	0.4958 0.549	
7		144367	248909	0.5606	
8					
9					
10		6013	10367	0.0233	

11	26755	46129	0.1038
12	47626	82114	0.1849
13	85533	147471	0.3321
14	109892	189470	0.4267

Cell Count

MS = 50 μ l

Avg. Count

Vol. contains
10⁶ Cells
(μ l)

12/20/99

	Cell Count	Avg. Count	Vol. contains 10 ⁶ Cells (μ l)
565 ± 59	1 521, 545, 567, 551	554.3	0.450
	2 552, 543, 541	545.3	0.458
	3 468, 455, 449	457.3	0.547
	4 649, 683, 666	666	0.375
	5 561, 523, 550	544.6	0.458
	6 605, 590, 603	599.3	0.417
	7 588, 574, 614	592	0.422
672 ± 76	8 750, 767, 739	752	0.332
	9 789, 772, 769	776	0.322
	10 721, 719, 710	716	0.348
	11 630, 666, 639	645	0.387
	12 612, 631, 645	629	0.397
	13 565, 574, 591	576	0.434
	14 638, 610, 580	609	0.410

TABLE-3

Expt. # : 1

Date/Time : 12/20/99

KBaf
cluster
[pCi/cell
x 148]

mBaf/cell
[pCi/cell
x
37]

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x 10 ⁶ Cells/ml]
1				
2				
3		457.3	1829200	0.0154
4		666	2664000	0.0506
5		544.6	2178400	0.2451 0.1117
6		599.3	2397200	0.2068
7		592	2368000	0.2367
8				
9				
10		716	2864000	0.008

0.57

1.87

4.13

7.65

8.76

0.30

1.57 11

2.72 12

5.34 13

6.48 14

645

629

576

609

2580000

2516000

2304000

2436000

0.0423

0.0734

0.1442

0.1751

2.29

7.49

~~16.30~~

30.61

35.04

~~19.20~~

~~5.95~~

~~10.87~~

~~21.33~~

~~25.92~~

12/29/99

1.2	155, 165, 142	}	145.16	
2.2	135, 129, 145			
3.2	49, 58, 68		58.33	0.4018
4.3	89, 101, 110		10.0	0.0688
5.4	58, 62, 75		0.68	0.0047
6.4	12, 15, 9		0.12	0.0008
7.4	5, 7, 9		0.07	0.00048
8.2	175, 185, 181	}	173.93	
9.2	169, 173, 160			
10.2	51, 56, 62		56.33	0.3241
11.3	112, 104, 119,		11.16	0.0642
12.3	75, 87, 64		7.53	0.0433
13.4	27, 35, 44		0.35	0.002
14.4	9, 14, 20		0.14	0.00082

12-183

3 HT22 Low; Expts #2-3
(MEHA only)

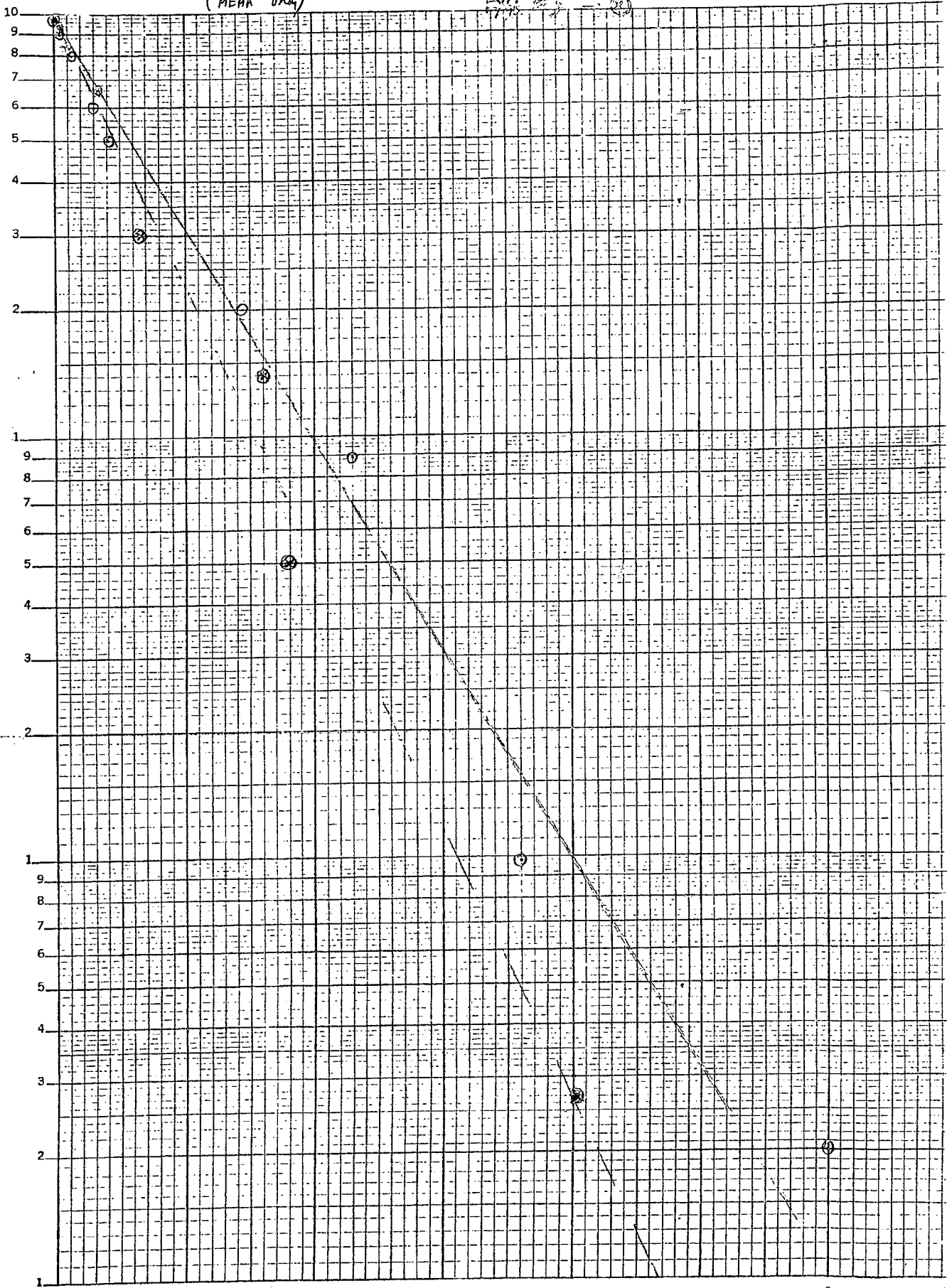
100% Abel

0.1

0.01

0.001

Semi-Logarithmic
3 Cycles x 10 to 10⁴ inch



0.02 0.04 0.06 0.08 0.1 0.12 psi/cell^{1/4}