

Exp. 4

P-32 + 5% DMSO at ~~48~~⁵⁰°F V79 Cells

6/27/95

Purpose: To determine the capacity of 5% DMSO to protect against chronic P-32 external irradiation.

- Procedure:
- ① Split the cells and make 400,000 cells/ml cell suspension.
 - ② ~~Split~~ Pipette 1 ml cell suspension into each culture tube and add DMSO and/or MEMA and/or Radiochemical according to Table 1.
 - ③ Roll the cells at 50°R in the refrigerator for 3 days.
 - ④ ~~Roll~~ Take out the tubes, keep them in ice add 8 ml MEMA spin the cells down, take 10 μ l medium for counting activities
 - ⑤ Wash the cells 4 times, ~~roll~~ resuspend in 2 ml medium, take 100 μ l cell suspension for counting activities bound to the cells.
 - ⑥ Make 3 serial dilutions (10^2 , 10^3 & 10^4 cells/ml) and plate the cells and incubate them at 37°C & 5% CO₂ & 95% Air in an incubator
 - ⑦ ~~Roll~~ Stain the cell colonies after 7 days and count them.

Table 1 :-

Radiochemical 2mCi NEX-053 P-32 orthophosphoric acid. 7/5/95 2 $\frac{mCi}{ml}$: 2.95 $\frac{mCi}{ml}$ 6/27

	MEMA w/10% DMSO (ml)	MEM (ml)	MEM 4×10^5 cells (ml)	Approx Dose (rad)	MCi P-32	μ l P-32	Dilutions to Plate	14.3d w/1 mCi/ml
1	1	0	1	0	0	0	10^2	
2	1	0	1	0	0	0	10^2	
3	1	0	1	500	11.75	4.0	$10^2, 10^3$	
4	1	0	1	1000	23.5	8.0	$10^2, 10^3, 10^4$	
5	1	0	1	1500	35.25	12.0	$10^3, 10^4$	
6	0	1	1	0	0	0	10^2	} Make Double dilutions to keep one in incubator directly and the other in Refrigerator.
7	0	1	1	0	0	0	10^2	
8	0	1	1	500	11.75	4.0	$10^2, 10^3$	
9	0	1	1	1000	23.5	8.0	$10^2, 10^3, 10^4$	
10	0	1	1	1500	35.25	12.0	$10^3, 10^4$	

Cell count: 1840, 1802 $\Rightarrow 7.28 \times 10^5$ cells/ml

$$\frac{400,000 \times 11}{7.28 \times 10^5} = 6.04 \text{ ml} \Rightarrow 4.4 \times 10^6 \text{ cells}$$

6 ml cells susp + 5 ml Medium $\Rightarrow 4 \times 10^5$ cells/ml

Group #	Cell counts	Avg	Total # of cells/ml.
1.	505, 474	490	1.96×10^5
2.	527, 477	502	2.00×10^5
3.	495, 474	485	1.94×10^5
4.	467, 458	463	1.85×10^5
5.	446, 497	472	1.89×10^5
6.	451, 448	450	1.80×10^5
7.	515, 485	500	2.00×10^5
8.	469, 458	464	1.85×10^5
9.	490, 465	478	1.91×10^5
10.	441, 435	443	1.77×10^5

Self Dose Calculation

$$S\text{-value } C \leftarrow C \text{ for P-32} = 2.46 \times 10^{-4} \frac{\text{Gy}}{\text{Bq} \cdot \text{s}}$$

$$= 2.46 \times 10^{-4} \frac{\text{Gy}}{\text{Bq} \cdot \text{s}} \left(\frac{100 \text{ rads}}{1 \text{ Gy}} \right) \left(\frac{1 \text{ Bq}}{27.02 \text{ Pci}} \right) \left(\frac{3600 \text{ s}}{\text{h}} \right)$$

$$= 3.28 \frac{\text{rads}}{\text{Pci} \cdot \text{h}}$$

Consider cell cycle time = 6h.

$\therefore T_e < 6\text{h}$ consider 6h.

$$T = 1.44 * T_e = 8.64 \text{ h}$$

Self Dose to the cell in the case of Group (E) i.e. 0.144 Pci/cell

$$= 3.28 \frac{\text{rads}}{\text{Pci} \cdot \text{h}} \times 0.144 \frac{\text{Pci}}{\text{Cell}} \times 8.64 \text{ h.}$$

$$= 4.08 \frac{\text{rads}}{\text{cell}}$$

NB: This Dose is negligible compared to the ~~est~~ estimated cross-dc during ~~incubation~~ incubation period which is equal to 1500 rads.

SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N R5232:N

M# 1 AGC:N GCF:N RCM:N

CHANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0

CHANNEL 2-LL: 0 UL: 670 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0

CHANNEL 3-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0

DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: G 1.00000

HAL LIFE(DAYS):N

600cpm 5ml

SAM	POS	CH	Gr#	CPM	2SIG%	TIME	EL TIME	AVG H#	Activity Added	Activity Measured	ERR	
1	28-1	1		17.00	46.51	1.00	1.47	92.0	Controls			
		2	①	26.00	39.22							
		3		34.00	34.30							
2	28-2	1		8.00	70.71	1.00	3.07	91.0	BG			
		2		12.00	57.74					0	0	
		3	②	17.00	48.51							
3	28-3	1		1675.00	4.89	1.00	4.63	92.0	11.75	12.21		
		2	③	8942.00	2.13							
		3		27150.00	1.21							
4	28-4	1		4007.00	3.16	1.00	6.21	92.0	23.5	23.1		
		2	④	18031.00	1.49							
		3		51346.00	0.88							
5	28-5	1		4353.00	3.03	1.00	7.78	91.0	35.25	26.88		
		2	⑤	20701.00	1.39							
		3		59722.00	0.82							
6	28-6	1		18.00	47.14	1.00	9.34	90.0	0	0		
		2	⑥	28.00	37.80							
		3		32.00	35.36							
7	28-7	1		21.00	43.64	1.00	10.89	90.0	0	0		
		2	⑦	26.00	39.22							
		3		30.00	36.51							
8	28-8	1		2083.00	4.38	1.00	12.45	90.0	11.75	13.04		
		2	⑧	11294.00	1.88							
		3		28998.00	1.17							
9	28-9	1		4339.00	3.04	1.00	14.03	91.0	23.5	25.0		
		2	⑨	21817.00	1.35							
		3		55853.00	0.85							
10	28-10	1		5670.00	2.66	1.00	15.66	91.0	35.25	36.85		
		2	⑩	31113.00	1.13							
		3		81884.00	0.70							

Medium Activity Before Wash:

Counted 10 µl after adding 8 ml to 8 ml Original cell suspension: ∴ original activity in tube = N x 1000

12	28-12	1		7.00	75.59	1.00	17.28	104.0	Controls	PC/cell		
		2	⑪	19.00	45.88							
		3		28.00	37.80							
13	28-13	1		14.00	53.45	1.00	18.82	107.0	0			
		2	⑫	24.00	40.82							
		3		30.00	36.51							
14	28-14	1		332.00	10.98	1.00	20.37	101.0	0.049			
		2	⑬	1103.00	6.02							
		3		2223.00	4.24							
15	28-15	1		549.00	8.54	1.00	21.92	103.0	0.089			
		2	⑭	1829.00	4.68							
		3		3979.00	3.17							

Activity in the Cells After Washes:

100 µl of cell suspension from each group was counted. which contains 20,000 cells ∴ Activity per cell = $\frac{N}{60 \times 0.037 \times 20,000}$

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR	
16	28-16	1	1139.00	5.93	1.00	23.48	103.0	0.144	
		2	⑮	3299.00	3.48				
		3	6439.00	2.49					
17	28-17	1	18.00	47.14	1.00	25.03	104.0	0	
		2	⑯	32.00	35.36				
		3	42.00	30.86					
18	28-18	1	15.00	51.64	1.00	26.64	104.0	0.023	
		2	⑰	22.00	42.64				
		3	32.00	35.36					
19	** 1	1	139.00	16.96	1.00	28.23	103.0	0.049	
		2	⑱	505.00	8.90				
		3	1059.00	6.15					
20	** 2	1	218.00	13.55	1.00	29.79	104.0	0.076	
		2	⑲	967.00	6.43				
		3	2247.00	4.22					
21	** 3	1	236.00	13.02	1.00	31.35	104.0		
		2	⑳	1332.00	5.48				
		3	3439.00	3.41					

10. per
1000

MC: .037 MB
MC: ()