

<sup>131</sup>IUDR from Pools of <sup>Synthesys</sup> ~~Exp~~ 1, 2, 3, followed by HPLC  
Solvent 90% H<sub>2</sub>O 10% MeOH

## Subcellular Distribution

4/18/97

<sup>131</sup>IUDR V79 cells

- Trypsinize cells, resusp in MEMB at  $\sim 10^6$ /ml
- 3 ml into 2 tubes at 5pm on roller, 37°C, 5% CO<sub>2</sub>
- 8pm Add <sup>131</sup>IUDR  $\sim 2\frac{1}{2}$   $\mu$ Ci per tube
- 9pm 4/12/97 Wash 3X cold calcium-free salt sol.
- Resusp in 3 ml sucrose
- Leave in refrigerator 5 min
- Add 3 ml sucrose w/ 2% Triton while vortexing
- Refrig: 2 min, vortex 30s
- Remove 2x 100  $\mu$ l for counting whole cell activity
- Spin remainder 15 min, 2000 rpm 4°C
- Decant cytoplasmic fraction into tube
- Remove 2x 500  $\mu$ l for counting cyto
- Wash nuclei 1X with 10  $\mu$ l sucrose
- Decant, resusp in 2 ml sucrose
- Add 4  $\mu$ l guanidine HCl
- Add 6  $\mu$ l EtOH
- Filter all 12  $\mu$ l through type A/E
- Wash with 12  $\mu$ l 1:1 EtOH: Guanidine HCl
- Count Filter

4/18/97

I-131<sup>a</sup> Subcellular Distribution  
Exp. 2 (After 2<sup>nd</sup> HPLC)

Counts in cells 100  $\mu$ l  $\Rightarrow$  5770 } Avg = 5690 CPM  
(6.3 ml) 5610 }

Total Activities in cell susp = 358470 CPM  
(6.3 ml)

Counts in cytoplasm 500  $\mu$ l  $\Rightarrow$  121 } Avg = 129 CPM  
137 }

Total Activities in cytoplasm = 1548 CPM. (0.43%)

Activities in the nucleus

= 357000

Activities in re DNA

= 233828 CPM

+  
3011

236839

Counts in Supernatant = 384 CPM.

$\therefore$  100% of re Nuclear Activity is bound to DNA

Lost counts verification:

Activities in tube + Pasteur pipette = 4253

<sup>131</sup>IUDR is HPLC of Paced Syn. 1-3  
Solvent 90% H<sub>2</sub>O 10% MeOH

4/17/97  
Thurs.

## Exp. 2

<sup>131</sup>

IUDR + 5% DMSO

- trypsinize cells, resusp in 400,000 cells/ml MEMB, 1 ml/tube
  - roll 4h at 37°C, 5% CO<sub>2</sub>, In roller at 3:00pm
  - Add activity according to table on following pg
- See TABLE
- Return to roller at 37°C, 5% CO<sub>2</sub> at 7:00pm

4/18/97

- Remove from roller, centri.f. 2000 rpm, 4°C, 10min
- Transfer 100µl of super to glass tube
- 3x 10µl onto tissues for medium determination
- Decant remaining
- Wash 3X with wash MEMA (ice cold)
- Resusp in MEMA ± 5% DMSO (ice cold)

Tubes 1-5 5% DMSO

Tubes 6-10 No DMSO

~~Return to roller at 10.5°C at 11:00 am 4/18/97~~

- Syringe 5X, Transfer 0.5 ml to glass tube
- 1x 100µl Coulter, 3x 100µl for cell activity
- Cap tightly
- Return to roller at 10.5°C at 11:00 am 4/18/97

$^{131}\text{I} \text{UdR} + 5\% \text{ DMSO}$

Exp. 2

04/17/97

This  $^{131}\text{I} \text{UdR}$  is after 2<sup>nd</sup> HPLC - very high purity, new conc

$$\begin{aligned} \text{Route in 10 mL of MEMB} &= \frac{27212}{0.175 \times 0.812 \times 2.22 \times 10^6 \times 10} \\ &= 8.63 \times 10^3 \frac{\mu\text{Ci}}{\mu\text{L}} \end{aligned}$$

Tube #	MEMB	MEMB $\pm$ I(31)
1	1	0
2	1	0
3	884 $\mu\text{L}$	116 $\mu\text{L}$
4	768 $\mu\text{L}$	232 $\mu\text{L}$
5	652	<del>464</del> $\mu\text{L}$
		•
6	1	0
7	1	0
8	884	116
9	768	232
10	652	<del>464</del>

0.72 mm Annulus

~~72 mm~~  
0.72 mm / 1.08 mm

0.72 mm



20 - 100  $\mu\text{L}$

645, 670

$^{131}\text{I}$ UDR + 5% DMSO

Exp. 2

(After 12 h roll with  $^{131}\text{I}$ UDR)

Cell Counts, Postwash

10:00am

4/18/97

1.	1206, 1253, 1312	1257	
2.	1201, 1257, 1241	1233	
3.	1043, 1094, 1048	1062	
4.	1188, 1136, 1138 }	1154	7.96
5.	1084, 996, 957 }	1012	15.06
6.	1270, 1255, 1224	1249.7	
7.	1266, 1169, 1214	1216.3	
8.	1159, 1164, 1144	1155.7	
9.	1227, 1155, 1181	1187.7	
10.	<del>1301</del> , 1211, 1173, 1176	1187	16.6

- 11:02 am 4/18/97

Transferred to rocker roller 11°C

4/18/97  
4pm $^{131}\text{IWR} + 5\% \text{ DMSO}$ I-131 - Counting After 12h roll

Counts in 10 $\mu\text{l}$ of Medium Area (CPM)	Aug $\pm$ Std	Estimated Activity/ $\mu\text{ml}$	Added Act. $\frac{\mu\text{Ci}}{\text{ml}}$
1. 1			
2. $\phi$			
3. 1144, 1136, 1174	1151 $\pm$ 20	0.365 $\mu\text{Ci}/\text{ml}$	0.5
4. 2185, 2289, 2312	2262 $\pm$ 68 (1.96)	0.717 $\mu\text{Ci}/\text{ml}$	1.0
5. 4197, 4327, 4278	4262 $\pm$ 92 (1.88)	1.35 $\mu\text{Ci}/\text{ml}$	2.0
6. 0			
7. 0			
8. 1044, 1111, 1066	1072 $\pm$ 35	0.34 $\mu\text{Ci}/\text{ml}$	0.5
9. 1978, 2072, 2031	2027 $\pm$ 47	0.643 "	1.0
10. 3960, 3916, 4038	3917 $\pm$ 62	1.24 "	2.0

Counts in 100 $\mu\text{l}$ of Cell suspension	Aug $\pm$ Std	Estimated Activity in cells (total 2ml)	Total Activity
1. 0			
2. 0			
3. 4073, 3931, 3963	3989 $\pm$ 75	0.252 $\mu\text{Ci}$ =	0.982
4. 9437, 9175, 8936	9182 $\pm$ 250	<del>0.582</del> 0.582 $\mu\text{Ci}$	2.016
5. 15304, 15213, 15207	15241 $\pm$ 54	0.966 $\mu\text{Ci}$	3.67
6. 0			
7. 0			
8. 4513, 4325, 4518	4452 $\pm$ 110	0.282 $\mu\text{Ci}$	0.962
9. 9497, 9332, 9569	9466 $\pm$ 122	0.600 $\mu\text{Ci}$	1.89
10. 19438, 19576, 20178	19731 $\pm$ 393	1.25 $\mu\text{Ci}$	3.73

4/21/97

Exp 2  
<sup>131</sup>IUdR + 5% DMSO

- Remove cells from 10.5°C, put on ice
- Centrifuge 2000 rpm 4°C, 10 min
- Decant
- Wash 2X with 10 ml wash MEMA (ice cold)
- Resusp in 2 ml wash MEMA
- Syringe 5X, 100µl Coulter count
- Serial Dilutions 0.5 ml → 4.5 ml
- Seed 25 cm<sup>2</sup> flasks
- 3x100µl cells for activity determination

Colonies

1.2	11, 3, 8
2.2	5, 5, 7
3.4	70, 66, 72
4.4	4, 2, 2
5.4	66, 66, 49
6.2	8, 9, 11
7.2	3, 10, 17
8.4	16, 16, 21
9.4	<del>6, 8, 8</del> 4
10.4	<del>6, 8, 8</del> 6, 8, 8

Exp 2  
<sup>131</sup>IUDR + 5% DMSO  
 Post 72 h Roll

4/21/97

Cell Counts			Aug ± std.
Tube 1	415, 418, 390		408 ± 15
2	220, 270, 242	leaked	244 ± 25
3	554, 597, 561	571	
4	521, 520, 526	522	
5	502, 504, 505	504	
6	514, 569, 550	544.3	
7	527, 565, 509	533.3	
8	527, 518, 505	516.7	
9	935, 976, 962	957.7	
10	596, 611, 569	592	

Activity Counts 100% washed cells 3 pm 4/21/97  
 GeLi, EFF = 0.176, Yield = 0.872

1	0		
2	0		
3	1622, 1741, 1662	1655	
4	3836, 3816, 3857	3836	7.3
5	6530, 6585, 6707	6607	13.1
6	0		
7	0		
8	1729, 1819, 1732		
9	3970, 4102, 3978		
10	8558, 8834, 9085	8825.7	14.9