

5. Keep the tubes in the roller for 3-4 h at 37°C, 5% CO<sub>2</sub>

Date/Time: 12/20/01 2 pm

6. Prepare MEMB containing radioactivity in hood

µl <sup>131</sup>IdU (Stock : 1202 µCi/µl on 12/13/01 11:00am) + ml MEMB

Manufacturer: US Lot #: Calibration:

Stock 1202 µCi/ml on 12/13/01 11:00am

12/20/01 4:30pm → 7 days 5½ h = 7.23 days

$$\text{Conc. @ 12/20 4:30pm} = 1202 \left( e^{-\frac{0.693(7.23d)}{8.02d}} \right) = 644 \frac{\mu\text{Ci}}{\text{ml}}$$

$$5 \text{ ml} \times 28 \frac{\mu\text{Ci}}{\text{ml}} = 140 \mu\text{Ci} \Rightarrow 0.277 \text{ ml stock } ^{131}\text{IdU} + 4.78 \text{ ml MEMB}$$

filter through 0.2 µm Acrodisc

7. After 3-4 h, add 1 ml MEMB to 1U-10U. To the other tubes 1-10, add MEMB with or without radioactivity according to Table below.

Tube #	<sup>131</sup> IdU µCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ <sup>131</sup> IdU [28 µCi/ml] (ml)
1	0	1.0	1.0	0
2	0	1.0	1.0	0
3	1	1.0	0.929	0.071
4	2	1.0	0.86	0.14
5	4	1.0	0.71	0.29
6	6	1.0	0.57	0.43
7	8	1.0	0.43	0.57
8	10	1.0	0.29	0.71
9	12	1.0	0.14	0.86
10	14	1.0	0	1.0

Make 5 ml

7. Return test tubes to roller for 12-14 h.

Date/Time: 4:30pm

8. Next day, while test tubes are in roller label 10 gamma-tubes (13 X 100 mm VWR glass test tube)

9. After ~12-14 h incubation period, remove all tubes and centrifuge at 2000 rpm at 4°C for 10 min (precooled centrifuge).

Date/Time: 7:00am 12/21/01

10. Remove buckets from centrifuge and carefully remove 150 µl of supernatant from tubes containing radioactivity and place in pre-labeled gamma-tubes.

11. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA