

Tube #	³ HTdR μCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ³ HTdR [60 μCi/ml] (ml)
1	0	1.0	1.0	0
2	0	1.0	1.0	0
3	2	1.0	0.933	0.067
4	5	1.0	0.83	0.17
5	7.5	1.0	0.75	0.25
6	10	1.0	0.67	0.33
7	15	1.0	0.50	0.50
8	20	1.0	0.33	0.67
9	25	1.0	0.20	0.80
10	30	1.0	0	1.0

Need 4 ml

250 μCi - 270 μCi

4.23
3.75 ml MEMB+ 0.27 ml ³HTdR

8 ml

Need 4.5 ml 9/27/01

7. Return test tubes to roller for 12-14 h. **Date/Time:** 7:25 pm
8. Next day, while tubes are in roller label 10 gamma-tubes (13 X 100 mm VWR glass tube)
9. After ~12-14 h incubation period, remove all tubes and centrifuge at 2000 rpm at room temp for 10 min. **Date/Time:** 9:30 am 9/28/01
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
12. Centrifuge tubes for 10 min at 2000 rpm
13. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm
17. Decant supernatant, click tubes, vortex, resuspend in 10 ml MEMA.
18. Centrifuge tubes for 10 min at 2000 rpm.
22. Decant supernatant completely, click tubes, vortex.
23. Transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 μl) using 200 μl pipette tip.
24. Again add 200 μl MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 μl)
25. Centrifuge tubes for 5 min at 1000 rpm, 4°C
26. Transfer tubes at 10.5°C for 72 h. **Date/Time:** 11:25 am 9/28/01
27. Transfer 30 μl supernatant in three sets of 7 ml scintillation vials and add 6 ml liquid scintillation cocktail (Ecoscint) from 150 ul supernatant removed earlier and count them for radioactivity **Date/Time:** 10:00 am 9/28/01