

Exp. wBs004

wBs Survival $^3\text{HTdR}$ 100% Label

10/15/04

- 10/11/01 Seed wBs into 225 cm² flask w/ 30 ml D medium
- 10/14/01 Feed wBs with 30ml D medium (cells confluent)
- 10/15/01 Remove media from T225 by suction (Passage 25)
- Wash 1X w/ 15 ml PBS PS
 - Add 2 ml trypsin, 37°C, 4 min
 - Hit flask, resuspend in 7 ml D medium
 - Syringe 7X 21G. 5cc.
 - Coulter count 100µl cells, 20µl Isotone II, 100 µl manometer
- 2192 2215 2210
- $2205.7 \times 400 \times 5 = 4.41 \times 10^6 / \text{ml}$
- Load T25 w/ 5ml D medium
 - Transfer 4×10^6 cells (0.91 ml) in flasks
 - Place in incubator at 37°C 2.5% CO₂
 - Add activity as below: stock $^3\text{HTdR}$ straight into flask
 ↳ 6ml in flask

3:45 pm

7:45 pm

<u>Tube #</u>	<u>$^3\text{HTdR}$ µCi/ml</u>	<u>Activity to Add</u>	<u>Volume $^3\text{HTdR}$</u>
1	0	0	0
2	0	0	0
3	0 1	6µCi	6µl
4	1 2	12µCi	12µl
5	2 3	18µCi	18µl
6	3 4	24µCi	24µl
7	4 5	30µCi	30µl
8	5 10	60µCi	60µl
9	10 15	90µCi	90µl
10	15 20	120µCi	120µl