

| Flask # | $^{3}\text{H}\text{TR}$<br>$\mu\text{Ci}/\text{ml}$ | Activity<br>to Add (KCl) | Volume $^{3}\text{H}\text{TR}$ |                            |
|---------|---|--------------------------|--------------------------------|----------------------------|
|         |   |                          | 1 KCl/ml                       | 1 $\mu\text{Ci}/\text{ml}$ |
| 1       | 0   | 0 KCl                    | 0                              | 0                          |
| 2       | 0   | 0 KCl                    | 0                              | 0                          |
| 3       | 0.5   | 5 KCl                    | 5                              | 5                          |
| 4       | 1   | 10                       | 10                             | 10                         |
| 5       | 2   | 20                       | 20                             | 20                         |
| 6       | 4   | 40                       | 40                             | 40                         |
| 7       | 6   | 60                       | 60                             | 60                         |
| 8       | 10  | 100                      | 100                            | 100                        |
| 9       | 15  | 150                      | 150                            | 150                        |
| 10      | 20  | 200                      | 200                            | 200                        |

- aspirated medium with pipet - saved 1 ml for counting
- 11/12/02 - 10:30am washed flasks 4X with 10 ml PBS
- add 1ml trypsin,  $37^{\circ}\text{C}$ , 3 min
  - Hit flasks, add 10 ml D-Med (Total 11ml)
  - Pipet up and down 4 or 5 times
  - Centrifuge tubes for 10 sec @ 2000 rpm,  $4^{\circ}\text{C}$ , decant, Vortex, brief resuspend in 5 ml D-Med
  - Syringe SX 21G 5cc
  - Coulter Count 100  $\mu\text{l}$  cells, 20 ml I-Stone II, 500 kD nanometer

|          |      |      |      |                              |         |             |
|----------|------|------|------|------------------------------|---------|-------------|
| 1) 2225  | 2118 | 2104 | 2149 | $0.86 \times 10^6/\text{ml}$ | 4.65 ml |             |
| 2) 2118  | 2050 | 2027 | 2065 | $0.83 \times 10^6$ "         | 4.84    |             |
| 3) 1973  | 2006 | 1981 | 1987 | 0.795 "                      | 5.0     |             |
| 4) 2350  | 2327 | 2285 | 2321 | 0.93 "                       | 4.31    |             |
| 5) 1952  | 1962 | 1968 | 1961 | 0.78 "                       | 5.1     |             |
| 6) 1831  | 1878 | 1856 | 1855 | 0.74 "                       | 5.4     | S.0 ml only |
| 7) 1894  | 1867 | 1888 | 1883 | 0.75 "                       | 5.31    | S.0 ml only |
| 8) 1916  | 1952 | 1966 | 1945 | 0.78 "                       | 5.1     | available   |
| 9) 2120  | 2056 | 2091 | 2089 | 0.84 "                       | 4.8     |             |
| 10) 1956 | 1973 | 2002 | 1977 | 0.79 "                       | 5.06    |             |

Transfer  $4 \times 10^6$  cells to a new tube according to the last column