Challenge

**HPRT mutant selection**

Date: 27-Sep-01
Experiment: V79, HTdR, 100 % cluster, Roger's exp. #6

| Sample # | Coulter count H | | | Coulter bckgr. | Coulter Mode (μl) | # of cells per ml | Cell susp. volume for 200 000 cells (ml) |
|---|---|---|---|---|---|---|---|
| 1 | 2545 | 2567 | 2587 | 5 | 100 | 5122667 | 0.039 |
| 2 | 2452 | 2471 | 2458 | 5 | 100 | 4910667 | 0.041 |
| 3 | 2429 | 2309 | 2446 | 5 | 100 | 4779333 | 0.042 |
| 4 | 2352 | 2353 | 2288 | 5 | 100 | 4652000 | 0.043 |
| 5 | 2350 | 2377 | 2367 | 5 | 100 | 4719333 | 0.042 |
| 6 | 2184 | 2096 | 2178 | 5 | 100 | 4295333 | 0.047 |
| 7 | 2133 | 2242 | 2163 | 5 | 100 | 4348667 | 0.046 |
| 8 | 2491 | 2436 | 2396 | 5 | 100 | 4872000 | 0.041 |
| 9 | 2286 | 2298 | 2245 | 5 | 100 | 4542667 | 0.044 |
| 10 | 2289 | 2281 | 2433 | 5 | 100 | 4658667 | 0.043 |

Protocol:
1. Wash T75 1-2x with PBS (no Ca++, no Mg++)
2. Trypsinize the cells (2 ml trypsin /T75, 2-3 min, RT)
3. Add 10 ml wash medium (wash MEMA) / T75 flask
4. Resuspend cells and transfere them in 15 ml conical tube.
5. Spin the cells down: 4-5 min, 2K rpm)
6. Aspirate supernatant, click tube to disperse pelet
7. Add 5 ml regular medium (MEMB/FCS10%) and resuspend the cells
8. Siringe the cells using 5 ml singe & 21G needle.
9. Count the cells using coulter couter: 100 μl cell susp. + 20 ml Isotone.
10. Plate 2 x 10(5) cells / P100 dish in 7 ml MEMA/FCS10% x 10 dishes/dose point
11. Transfere dishes into incubator and let the cells to attach (2-4 hrs)
12. Take 2 x 10(5) cells suspension, make serial dilution (2 x 10x) to obtain 200 cells/ml.
13. Plate 200 cells / P 60 x 3 dishes / dose point for Plating Efficiency.
14. Repaet staep 11 and 12 for each sampling point.
15. After 2-4 hrs add 3 ml MEMA + 6-TO (60 μg 6-TO) into each P 100.
    (Final concentartion for 6-TO = 6 μg/ml) 6-T0 Stock sol. = 60 μg/ 3 ml MEMA)
16. Keep the dishes in standard culture condition for 8-10 days.
17. Wash HPRT- colonies 1-2 times with PBS, fix them with MetOH and stain.
18. Count colonies.