

Exp. wBs 003

- 10/2/01 - For last tube, click, vortex, resusp in 2ml Dmedium
Control - Syringe 5X 5ul 2IG
 - Coulter count 100ul cells in 20ul Isotone II
 3697 3638 3626
 $3653.7 \times 400 = 1.46 \times 10^6 / \text{ml}$
 - Perform 4 10X serial dilutions w/ PBS PS
 0.5ml \rightarrow 4.5ml
 - Transfer 1ml of final dilution (146 cells)
 into two T25 flasks with 3.5 ml Dmedium

- 10/3/01 - Process one 10.5°C capped
 2:30pm one 37°C capped
 one 37°C 25% CO₂ as follows

- ⚡ Prepare three 14ml tubes w/ 10ml PBS PS
 Remove Dmedium from 400ul tube
 Vortex tube, transfer cells w/ sterile pester pip.
 Wash 400ul tube 2X w/ 200ul PBS & transfer
~~Syringe 37°C 2.5% CO₂ tube~~
 Vortex, centrifuge 1350 rpm 10min room temp
 Decant, click, vortex, resusp in 2ml PBS PS
 Syringe 37°C 2.5% CO₂ 5X 2IG 3cc
 - observe in scope (clumps of 4 or 5 common)
 Add 0.5ml Accutase to each tube, set 15 min Room temp.
 - syringe 5X, (few clumps still)