

10/3/01

37°C 2.5% CO<sub>2</sub> : t = 1d

Coulter 100µl cells + 200µl Isotone II

1969 2001 1917 1962 × 400 = 7.85 × 10<sup>5</sup> /ml

Dilute 3 10X gives 785 /ml

Seed 0.25 ml of 3<sup>rd</sup> dilution for 200 cells10.5°C : t = 1d :

Syringe 5x 21G 3 ml (Accutase as above but longer)

Still some doublets with fair frequency (too many)

3092 3129 3088 3103 × 400 1.24 × 10<sup>6</sup> /ml

Dilute 4 × 10X gives 1.24 /ml

Seed 1.61 ml of 4<sup>th</sup> dilution for 200 cells37°C t = 1d :

Syringe (still too many doublets)

2434 2484 2260 2393 × 400 = 9.57 × 10<sup>5</sup> /ml

Dilute 3 × 10X gives 957 /ml

Seed 0.21 ml of 3<sup>rd</sup> dilution for 200 cells

\* Conclusion from above : 20% Accutase insufficient  
to break up clumps