

PREPARATIONS FOR CELL SEPARATIONS

B-cells – Ig bearing cells – are membrane stained and separated by machine (see following page)

MATERIALS FOR CELL SEPARATOR PERSONNEL

- ALL STERILE:
1. Siliconized 12 ml conical centrifuge tubes with beaded lip (for collecting fractions). Add to each at time of run .5 cc filtered inactivated FCS and .05 ml Gentamicin @ 10 mg/ml
 2. 10 x 75 mm tubes (for samples)
 3. Pasteur pipettes (for putting cells into sample tubes).
 4. Heat inactivated filtered F__ (for collecting tubes).
 5. 2 liters IX D's PBS filtered with IX penicillin and streptomycin (100 ug/ml) No FCS.
 6. Sterile glutaraldehyde fixed chicken R B cells.

SAMPLE: 1-2 x 10⁷ cells/ml D's PBS + 5% FCS (heat inactivated) penicillin & strep.

CALCULATIONS:

1. Cells come off machine @ 1.1×10^6 therefore a 12 ml tube = 1.6×10^7 cells
2. Flow rate is ~2 ml/hr therefore for 40% (+) cells @ 10^7 cells/ml (4×10^6 (+) cells/ml), a run = 8×10^6 (+) cells/hour. ~ tube/hour
3. 2×10^8 cells can be separated per long day.