

## HUMAN WHITE CELL SEPARATION (Wolfgang Kreth)

1. Cells received @  $1 \times 10^6$  cells/ml McCoy's + 25% AB+ serum.
2. Wash 2X in D's PBS to remove human serum.. (can be resuspended in McCoy's + 25% FCS)
3. Stain for 15 min @ 4°C. Fl\* $\alpha$ .Cohen fraction II @ 1/5
4. Wash 2X with 5 cc FCS gradients
5. Pass through 1-2 cc glass wool if cells are clumped.
6. Resuspend @  $1-2 \times 10^7$  cells/ml D's PBS + 5% FCS for application to Cell Separator machine (check smear in microscope)
7. Separation conditions:  
if 20% cells stained ("B" cells), separate 15-20% with the highest fluorescence, discard 25% dimmest cells, and collect 50-60% non fluorescent cells ("T" cells).
8. Resuspend @  $1 \times 10^6$  cells/ml McCoy's.
9. Determine purity of separated suspensions by microscope.

Clumping may be eliminated by treating cells for 3 min @ room temp with 1/5 of frozen DNase to 2.5 mg/ml