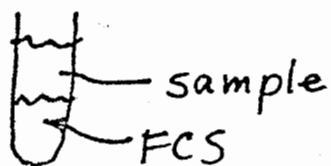


# FCS LABELING IN SUSPENSION

5/9/74  
Mike Hatfield  
7-6441

CELL LINE - C57 BLACK RadLV ( $F_L$ )  
ANTIGEN - GS ANTIGEN (MOUSE)  
ANTIBODY -  $\alpha$ -MuLV SERUM (RAT)  
LABEL - FITC-conjugated goat  $\alpha$ -rat (HYLAND)

1. Trypsinize cells in 0.2% trypsin, 5-10'
2. Neutralize in 10% FCS
3. Spin down pellet 1020 RPM, 10'
4. FIX for 10' in cold 50% acetone/~~100%~~ 1.6% NaCl
5. Spin, rinse 1X in 1.6% NaCl
6. Resuspend in appropriate amt. of  $\alpha$ -MuLV serum, diluted in 1.6% NaCl (typical 1:32)
7. Carefully transfer sample to another test tube containing 2-3 mls of 100% FCS; sample should form a bilayer:



8. Spin <sup>pellet</sup>
9. Resuspend in 1:32 titer goat  $\alpha$ -rat fl\*  
37°C, 30', w/ shaking (these conditions apply to (6))
10. Repeat step 7
11. Rinse 1X in 1.6% NaCl

NOTE - all sol'ns. should be filtered to remove dust, etc.