

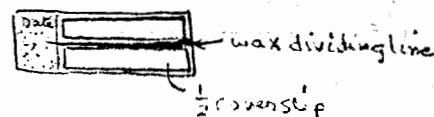
Cytoplasmic Fluorescent Antibody Staining

reagent F/P ratio ≈ 1

1. Remove cells, pass through glass wool to remove adherent cells, and wash twice in Iso MEM or Dulbecco's PBS containing 5% FCS. (RBC's may be lysed with hemolytic Gey's solution, but this usually creates debris which picks up stain).
2. For smears, pellet cells in a centrifuge tube and add one drop of FCS such that there are equal volumes of cell pellet and FCS.
3. Resuspend cells gently, place a small drop at the end of a microscope slide, and draw across with another slide.
4. For cytocentrifuge slides, resuspend cells at $1-2 \times 10^6$ cells/ml, and place 0.2 ml of the suspension in a cytocentrifuge block mounted in the cytocentrifuge with filter paper and microscope slide. Spin at 80 speed for 10', and then remove slide carefully. (70)
5. Air dry smears and cytocentrifuge slides, and fix in 95% ethanol for 15'.
6. Place one drop of fluorescent antibody reagent ^{@ ≈ 5 mg/ml - 1.5 mg/ml} on smear or cytocentrifuge pellet and cover with cover slip to spread out reagent.
7. Incubate 45' at room temperature in moist atmosphere.
8. Wash off cover slip with PBS from a squeeze bottle. Do not allow to dry.
9. Wash in Coplin jar in PBS for 10'.
10. Fix in 95% ethanol for 15'.
11. Air dry briefly.
12. Place 2 drops of 9:1 glycerol-PBS ^{pH 8.5} on smear (1 drop on cytocentrifuge slide) and cover with cover slip ^(22x50mm).
13. Seal with clear nail polish.
14. Unstained slides may be stored in a moist slide box in the refrigerator for several days.

To facilitate rapid counting, add fluorescent fixed CRBC in ratio of $1/10^3$ or $1/10^2$ lymphs and wbc.

if too few slides are available, bisect the slide along its length with a wax crayon and use 22x50mm coverslips which have been broken lengthwise. This way 2 effective slides are formed, and 2 reagents can be used to stain.



Staining Inside Fixed Cells