

Membrane Fluorescent Antibody Staining

request F/p ratio 2-3

1. Remove cells, pass through glass wool to remove adherent cells, lyse RBC's (not necessary) with hemolytic Gey's solution and wash once in Iso MEM or Dulbecco's PBS containing 5% FCS.
2. Count in hemacytometer.
3. Pellet $1 - 2 \times 10^7$ cells in a 3 ml conical glass centrifuge tube for staining. (For fewer cells ($\geq 5 \times 10^6$) 1-2 drops of a 5% suspension of glutaraldehyde-fixed chicken RBC's can be added as carrier cells.)
4. Remove supernatant.
5. Resuspend cell pellet in 100λ fluorescent antibody reagent.
6. Incubate at 4°C for 20', mixing after 15'.
7. Place cells on top of 1.5 ml ^{heat inactivated} FCS in a 3 ml conical tube, washing out the staining tube with 100λ medium and adding this to the cells. (absorb mouse cells if cytotoxic)
8. Centrifuge in the cold, and wash once more in cold medium.
9. If a second staining step is required, resuspend again in 100λ reagent and repeat.
10. For live mounts and smears, after the final wash add one drop of FCS to the pellet.
11. Resuspend cells gently. One drop of this can be mounted under a cover slip for live mounts.
12. For smears, place one drop at one end of a microscope slide, and draw across with another slide.
13. For cyto centrifuge slides, resuspend cells at $1 - 2 \times 10^6$ cells/ml, and place 0.2 ml of the suspension in a cyto centrifuge block mounted in the cyto centrifuge with filter paper and microscope slide. Spin at speed 80 for 10', and then remove slide carefully.
14. Air dry smears and cyto centrifuge slides.
15. Fix smears and cyto centrifuge slide in 95% ethanol for 15'. (5'-36 hrs)
16. Remove from ethanol and ^{forced} air dry. (quick dry & Fan)
17. Place 2 drops of 9:1 glycerol-PBS on smear (1 drop on cyto centrifuge slide) and cover with cover slip. - Tris pH < 9.0 < .01M
18. Seal with clear nail polish.
19. Unmounted slides may be stored in a moist slide box in the refrigerator for several days.

.2% NaN_3 can be used to stabilize membrane after 1^o stain when double staining.

20 millimolar NaN_3 prevents capping of AgAb complexes.