MINIMIZING BACKGROUND IN DIRECT AND INDIRECT IMMUNOFLUORESCENCE PROCEDURES

I. Titer the fluorochrome-conjugated antibody. If it is too concentrated, background may increase due to an increase in non-specific interactions.

II. BEFORE applying the primary antibody, incubate with excess protein such a bovine serum albumin (BSA), non-fat dry milk, or, for staining protocols with secondary antibodies, normal serum from the same host as the labeled secondary antibody. This step may reduce background by blocking non-specific interactions between the primary antibody and the cell surface or intracellular structures.

III. For indirect staining protocols, AFTER applying the primary antibody, incubate with 5% to 10% normal serum from the same host as the labeled secondary antibody. This step may reduce unwanted interactions between the secondary antibody and the primary antibody, the cell surface, or intracellular structures.

It is possible to avoid this step by diluting the labeled antibody with normal serum from the same species. This works well in many cases, but occasionally it can lead to the formation of immune complexes between the labeled secondary antibody and immunoglobulin in the normal serum. These complexes may bind to some cellular structures preferentially, or they may eventually lead to a loss of desired antibody activity.

IV. Use F(ab')2 fragments where background may be due to binding of the whole molecules of primary or secondary antibodies to Fc-receptors. F(ab')2 fragments of most secondary antibodies are readily available. F(ab')2 fragments of primary antibodies, however, are either unavailable or difficult to produce. Alternatively, incubate fresh tissues or cells with normal serum in the presence of NaN3 prior to addition of the primary antibody. Under these conditions, background due to Fc-receptor binding is not significant even if whole antibody molecules were used in subsequent steps.

V. Background also can arise from unwanted cross-reactivity between labeled antibodies and any other immunoglobulin inherent or added to the experimental system. To reduce this background, all labeled antibodies should be absorbed against other species to reduce binding to those immunoglobulin introduced from culture fluids, present on cell surfaces or in tissues, or added as primary and other secondary antibodies during multiple labeling. Cross-reactivity between multiple primary and secondary antibodies during multiple labeling also can be reduced by using labeled antibodies which have been derived from a single host species as well as absorbed against other host species.