



The Power to Quantify

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## Immunofluorescence Staining Protocol



- Prepare slides as described above for immunoperoxidase staining, omitting the final step involving treatment of cells with  $H_2O_2$ .
- Use suction to remove reagents after each step, but avoid drying of specimens between steps. Use sufficient reagent to cover the specimen (approximately 100–500  $\mu$ l per slide is adequate).
- Incubate specimens with 10% normal blocking serum ([Normal Sera for Immunohistochemistry](#)) in PBS ([Buffers and General Solutions](#)) for 20 minutes to suppress non-specific binding of IgG. Blocking serum ideally should be derived from the same species in which the secondary antibody is raised. Wash with PBS.
- Incubate with primary antibody for 60 minutes. Optimal antibody concentration should be determined by titration; recommended range is 0.5–5.0  $\mu$ g/ml in PBS with 1.5% normal blocking serum. Wash with three changes of PBS for 5 minutes each.
- Incubate for 45 minutes with either biotin-conjugated or fluorochrome-conjugated secondary antibody ([Secondary Antibodies for Immunohistochemistry](#)) diluted to 1–5  $\mu$ g/ml in PBS with 1.5%–3% normal blocking serum. Optimal antibody concentration should be determined by titration. Wash with three changes of PBS. If fluorochrome-conjugated secondary antibody is used, incubate in a dark chamber and omit the next step.
- Incubate with streptavidin-fluorescein for 15 minutes in a dark chamber. Optimal streptavidin conjugate concentration for a given application should be determined by titration; recommended range is 10–20  $\mu$ g/ml in PBS. Wash extensively with PBS.
- Mount coverslip with aqueous mounting medium or 90% glycerol in PBS.
- Examine using a fluorescence microscope with appropriate filters. Store slides in a dark location at room temperature (UltraCruz™ Mounting Medium: [sc-24941](#)) or at 4° C (glycerol/PBS mount).