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IMMUNOCYTOCHEMISTRY (ICC)

This immunocytochemistry protocol should be used as a guide for each researcher to build their own protocol, as each reagent will need to be optimized for use in the particular species, tissue type and application combination.

Reagents Required

1) PBS Wash Buffer. Phosphate Buffered Saline (PBS). Use 10x PBS, pH 7.2 (0.2M Potassium Phosphate, 1.5M NaCl). Dilute appropriate volume to 1x with de-ionized water.
2) Formaldehyde Fixative. Dilute to 4% in PBS buffer.
3) Antibody Dilution Buffer. Prepare 100ml of PBS wash buffer supplemented with 1ml of normal serum of same species as the host for the secondary antibody.
4) Biotinylated Secondary Antibody. Prepare dilution of biotinylated secondary antibody in Antibody Dilution Buffer. Use biotinylated secondary antibody conjugate against the same species as the primary antibody.
5) Streptavidin Peroxidase. Prepare dilution of Streptavidin Peroxidase in PBS buffer.
6) DAB Substrate.
7) Hematoxylin Counterstain and Mounting Media

Procedure

1) Rinse sections in PBS-Tween 20 for 2 x 2 minutes.
2) Serum Blocking: incubate sections with normal serum block – species same as secondary antibody, for 30 minutes to block non-specific binding of immunoglobulin. Note: since this protocol uses avidin-biotin detection system, avidin/biotin block may be needed based on tissue type. If you do require this, the avidin/biotin block should be done after the normal serum block.
3) Primary Antibody: incubate sections with primary antibody at appropriate dilution in primary antibody dilution buffer for 1 hour at room temperature or overnight at 4 °C.
4) Rinse in PBS-Tween 20.
5) Secondary Antibody: incubate sections with biotinylated secondary antibody at appropriate dilution in PBS for 30 minutes at room temperature.
6) Rinse in PBS-Tween 20 for 3 x 2 minutes.
7) Detection: incubate sections in FITC-Avidin D in PBS for 30 minutes at room temperature. Protecting slides from light starting from this step to the end by covering slides with aluminum foil or black box.

8) Rinse in PBS-Tween 20 for 3 x 2 minutes.
9) Counterstain with PI or DAPI if desired.
10) Rinse in PBS-Tween 20.
11) Dehydrate through 95% ethanol for 2 min, 100% ethanol for 2 x 3 minutes.
12) Coverslip with anti-fade mounting medium.