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## Fluorescence Detection of Fluorescein-Labeled ISH Probes

1. After hybridization of labeled DNA/RNA probes, block tissue sections or chromosome spreads for 30 minutes in 1x ISH blocking solution (5x ISH Blocking Solution, Cat. No. MB-1220). The effectiveness of the blocking solution may be enhanced by pre-warming the solution to 37 °C and incubating tissue sections/chromosome spreads for 30 minutes or longer at 37 °C.

*Note: 5% nonfat dry milk plus 0.1% Tween 20 in 4x SSC can be used as an alternative blocking solution. (4x SSC is 0.6 M NaCl, 60 mM sodium citrate, pH 7.0.) However, non-fat dry milk can contain variable amounts of biotin which could reduce staining if used as a diluent for (strept)avidin conjugates.*

2. Dilute each of the detection reagents, Biotinylated Anti-Fluorescein, (Cat. No. BA-0601) and Fluorescein Avidin DCS (Cat. No. A-2011), to 10 µg/ml in 1x blocking solution for approximately 30 minutes before use to further reduce any non-specific binding.

3. Tip off the blocking solution and incubate with Biotinylated Anti-Fluorescein solution (10 µg/ml) for 30 minutes at room temperature.

4. Wash slides for 2 x 3 minutes in blocking solution.

5. Incubate with the Fluorescein Avidin DCS solution (10 µg/ml) for 30 minutes at room temperature.

6. Wash slides 2 x 5 minutes in 4x SSC + 0.1% Tween 20 before coverslipping with any one of the following mounting media: Vectashield Mounting Medium (Cat. No. H-1000), Vectashield Mounting Medium with DAPI (Cat. No. H-1200), Vectashield Mounting Medium with propidium iodide (Cat. No. H-1300), Vectashield HardSet Mounting Medium (H-1400), or Vectashield HardSet Mounting Medium with DAPI (Cat. No. H-1500).

*For additional guidelines on the enzymatic or fluorescent detection of ISH probes, please request the "In Situ Hybridization Detection Systems" brochure or visit our website.*