



---

## Fluorescence Detection of Fluorescein-Labeled ISH Probes

1. After hybridization of labeled DNA/RNA probes, block tissue sections or chromosome spreads for  $\geq 30$  minutes in 1x ISH blocking solution (5x ISH Blocking Solution, 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<sup>®</sup> 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 (BA-0601) and Fluorescein Avidin DCS (A-2011) to 10  $\mu\text{g/ml}$  in 1x blocking solution for approximately 30 minutes before use to minimize any non-specific binding.

3. Drain off the blocking solution from the specimen and incubate with Biotinylated Anti-Fluorescein solution (10  $\mu\text{g/ml}$ ) 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  $\mu\text{g/ml}$ ) for 30 minutes at room temperature.

6. Wash slides 2 x 5 minutes in 4x SSC + 0.1% Tween<sup>®</sup> 20 before coverslipping with any one of the following mounting media: VECTASHIELD<sup>®</sup> (H-1000), VECTASHIELD<sup>®</sup> with DAPI (H-1200), VECTASHIELD<sup>®</sup> with propidium iodide (H-1300), VECTASHIELD<sup>®</sup> Hard+Set<sup>™</sup> (H-1400), VECTASHIELD<sup>®</sup> Hard+Set<sup>™</sup> with DAPI (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: [www.vectorlabs.com](http://www.vectorlabs.com).*

*Tween<sup>®</sup> 20 is a registered trademark of Atlas Chemical Industries*