



Chromogenic 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 Alkaline Phosphatase Anti-Fluorescein (Cat. No. MB-2100) to 5 µg/ml in 1x blocking solution approximately 30 minutes before use to further reduce any non-specific binding.

3. Tip off the blocking solution and incubate with Alkaline Phosphatase Anti-Fluorescein solution (5 µg/ml) for 30 minutes at room temperature.

4. Wash slides for 2 x 3 minutes in 100 mM Tris, pH 9.5 buffer.

5. Visualize the stain by incubating the tissue section or chromosome spread in BCIP/NBT substrate working solution prepared according kit instructions (BCIP/NBT Substrate Kit, Cat. No. SK-5400). Incubate until desired sensitivity is achieved.

Note: For an overnight incubation in the BCIP/NBT substrate solution, use the Alkaline Phosphatase Anti-Fluorescein reagent at a concentration of 0.2 – 2.0 µg/ml).

6. Wash in 100 mM Tris, pH 9.5 buffer for 5 minutes.

7. Rinse in tap water and counterstain if desired (BCIP/NBT substrate is compatible with Vector Nuclear Fast Red counterstain, Cat. No. H-3403, and Vector Methyl Green, Cat. No. H-3402).

8. For permanent mounting, dehydrate, clear, and mount sections in Vectamount Mounting Medium (Cat. No. H-5000) which minimizes crystal formation in mounted sections.

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.