

Chromogenic Detection of Biotin-Labeled ISH Probes

1. After hybridization of labeled DNA/RNA probes, block tissue sections or chromosome spreads for ≥ 30 minutes in a blocking solution such as Casein Solution (Cat. No. SP-5020-250) or Bovine Serum Albumin, Immunohistochemical Grade (Cat. No. SP-5050-500). 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 min or longer at 37°C.
Note: 5% nonfat dry milk plus 0.1% Tween® 20 in 4x SSC (4x SSC is 0.6 M NaCl, 60 mM sodium citrate, pH 7.0.) can be used as an alternative blocking solution. 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 Streptavidin, Alkaline Phosphatase (Cat. No. SA-5100) to 1–5 µg/ml in 1x blocking solution approximately 30 min before use to further reduce any non-specific binding.
3. Tip off the blocking solution and incubate with Streptavidin, Alkaline Phosphatase solution (1–5 µg/ml) for 30 min at room temperature.
4. Wash slides for 2 x 3 min 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 to 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 Streptavidin, Alkaline Phosphatase reagent at a concentration of 0.3–0.5 µg/ml).
6. Wash in 100 mM Tris, pH 9.5 buffer for 5 min.
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® Permanent 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 see our website for a comprehensive listing of detection reagents.