

Fluorescence Detection of Biotin-Labeled ISH Probes

This procedure uses successive rounds of Fluorescein-Avidin DCS and Biotinylated Anti-Avidin to detect and amplify in-situ hybridization signals. The multiple binding capacities of Biotinylated Anti-Avidin D gives the capability to provide significant amplification. This antibody binds to Avidin D through the antigen binding sites or through the biotin residues that are covalently attached to the molecule. Following the first application of Fluorescein Avidin DCS, the signal is amplified by incubation with Biotinylated Anti-Avidin, followed by a second incubation with Fluorescein Avidin DCS. This procedure results in the introduction of several more fluorochromes at the target site.

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 each of the detection reagents, Fluorescein Avidin DCS (Cat. No. A-2011) and Biotinylated Anti-Avidin (Cat. No. BA-0300), to 5 µg/ml in 1x blocking solution approximately 30 min before use to further reduce any non-specific binding.

Note: This procedure will require twice the volume of Fluorescein Avidin DCS to Biotinylated Anti-Avidin.

3. Tip off the blocking solution and add the Fluorescein Avidin DCS solution (5 µg/ml). Incubate for 30 min at room temperature.
4. Wash slides for 2 x 3 min in blocking solution.

Note: If satisfactory sensitivity has been achieved, skip to step 8. For increased sensitivity, continue with steps 5 through 7.

5. Incubate with the Biotinylated Anti-Avidin solution (5µg/ml) for 30 min at room temperature.
6. Wash slides for 2 x 3 min in blocking solution.
7. Follow with a second incubation of the same Fluorescein Avidin DCS solution (5 µg/ml) for 30 min at room temperature.
8. Wash slides 2 x 5 minutes in 4x SSC + 0.1% Tween 20 before coverslipping with any one of the following mounting media: VECTASHIELD Vibrance® (Cat. No. H-1700), VECTASHIELD Vibrance® with DAPI (Cat. No. H-1800), VECTASHIELD® PLUS (Cat. No. H-1900), and VECTASHIELD® PLUS with DAPI (Cat. No. H-2000).

For additional guidelines on the enzymatic or fluorescent detection of ISH probes, please see our website for a comprehensive listing of detection reagents.