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 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 ºC and incubating tissue sections/chromosome spreads for 30 minutes or longer at 37 ºC.

Note: 5% nonfat dry milk plus 0.1% Tween 20 in 4x SSC can be used as an alternative blocking solution. (4x SSC i s 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, 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 minutes 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 minutes at room temperature.

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

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 minutes at room temperature.

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

7. Follow with a second incubation of the same Fluorescein Avidin DCS solution (5 µg/ml) for 30 minutes 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 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.