SUPPLEMENTAL PROTOCOL

Fluorescence Detection of Fluorescein-Labeled ISH Probes

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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, Biotinylated Anti-Fluorescein (Cat. No. BA-0601) and Fluorescein Avidin DCS (Cat. No. A-2011) to 10 µg/ml in 1x blocking solution for approximately 30 min 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 µg/ml) for 30 min at room temperature.
4. Wash slides for 2 x 3 min in blocking solution.
5. Incubate with the Fluorescein Avidin DCS solution (10 µg/ml) for 30 min at room temperature.
6. 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.

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