

Detection and amplification of FISH signal

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Equipment and reagents

- ◆ Fluorescence microscope (epifluorescence illumination), with suitable fluorescence objectives and filter sets (usually need separate filter sets for FITC, Texas red/rhodamine, and DAPI/AMCA, as well as a double or triple filter block)
- ◆ Moist chamber for antibody detection steps: use a plastic microscope slide box (Raymond Lamb) containing moist tissue paper (wring out excess water), placed in incubator or floated in water-bath. Alternatively, use metal trays (Lamb's immuno slide staining trays, Raymond Lamb) for both hybridization and detection steps.
- ◆ Avidin DCS-FITC (1 mg/ml) (Vector Laboratories)
- ◆ Biotinylated anti-avidin D (0.5 mg/ml) (Vector Laboratories)
- ◆ Propidium iodide (Sigma)
- ◆ 4', 6-diamidino-2-phenylindole (DAPI) (Sigma)
- ◆ Vectashield mountant (Vector Laboratories)
- ◆ Avidin DCS-Texas red (2.5 mg/ml stock) (Vector Laboratories)
- ◆ Diluent for antibodies: blocking solution (see [FISH hybridization and subsequent washes](#)), filtered through a 0.45 µm syringe filter—stock antibody solutions are stored at -20 °C
- ◆ Monoclonal anti-digoxigenin (Sigma)
- ◆ Rabbit anti-mouse Ig-FITC (Sigma)
- ◆ Monoclonal anti-rabbit-FITC (Sigma)

A. Biotin labelled probes

- 1 Dilute 2.5 µl of stock avidin DCS-FITC in 1 ml blocking solution (5 µg/ml final concentration). Use 100 µl of this under a 24 x 50 mm coverslip. Incubate in a moist chamber at 37 °C for 30 min.
- 2 Flick off coverslips and wash slides three times (for 3 min each) in wash solution (see [FISH hybridization and subsequent washes](#)).
- 3 Dilute 10 µl of stock biotin anti-avidin D in 1 ml blocking solution (5 µg/ml final concentration). Use 100 µl of this under 24 x 50 mm coverslip. Incubate in a moist chamber at 37 °C for 30 min.
- 4 Flick off coverslips and wash slides three times (for 3 min each) in wash solution.

- 5 Add 100 μl avidin-FITC (same as the first layer). Incubate for 30 min under coverslip as before.
- 6 Carry out the following final washes:
 - Wash once for 3 min in wash solution.
 - Wash twice (5 min each) in PBS.Dehydrate slides through an ethanol series. Air dry.
- 7 Mount slides in 40 μl Vectashield containing 1.5 $\mu\text{g/ml}$ 4',6-diamidino-2-phenylindole (DAPI) and 0.75 $\mu\text{g/ml}$ propidium iodide (PI) under a 24 x 50 mm coverslip. Seal the edges of the coverslip with rubber solution or nail varnish. The signal keeps well for several weeks if slides are stored at 4 $^{\circ}\text{C}$.

B. Digoxenin labelled probes

- 8 Prepare all antibody dilutions in blocking solution, filtered before use.
Make up the following antibody dilutions in 1 ml blocking solution:
 - 1st layer: 1.5 μl mouse monoclonal anti-digoxigenin.
 - 2nd layer: 1 μl rabbit anti-mouse-FITC.
 - 3rd layer: 10 μl monoclonal anti-rabbit-FITC.
- 9 Incubate sequentially in each antibody layer (100 μl under a 24 x 50 mm coverslip) for 30 min at 37 $^{\circ}\text{C}$ in a moist chamber. After each antibody layer, wash three times (3 min each) in wash solution.
- 10 Carry out the following final washes:
 - Wash once for 3 min in wash solution.
 - Wash twice (5 min each) in PBS.Dehydrate slides through an ethanol series. Air dry.
- 11 Mount in Vectashield containing 1.5 $\mu\text{g/ml}$ DAPI and 0.75 $\mu\text{g/ml}$ propidium iodide.

C. Dual colour detection of biotin and digoxigenin labelled probes

- 12 Prepare all antibody dilutions in blocking solution, filtered before use.
Make up the following antibody dilutions in 1 ml blocking solution:
 - 1st layer: 1 μl avidin-Texas red plus 1.5 μl mouse monoclonal anti-digoxigenin.
 - 2nd layer: 10 μl biotin-anti-avidin plus 1 μl rabbit anti-mouse-FITC.
 - 3rd layer: 1 μl avidin-Texas red plus 10 μl monoclonal anti-rabbit-FITC.

- 13** Incubate in each antibody layer for 30 min at 37 °C in a moist chamber. After each antibody layer, wash three times (3 min each) in wash solution.
- 14** Carry out the following final washes:
- Wash once for 3 min in wash solution.
 - Wash twice (5 min each) in PBS.
- Dehydrate slides through an ethanol series. Air dry.
- 15** Mount in Vectashield containing 1.5 µg/ml DAPI only.