fluorescent in situ hybridization protocol

Basically this protocol uses a sequential tyramide-based amplification (NEN Life Science Products) using peroxidase activity through TY-Biotin followed by ABC-Elite (POD) (Vector Labs), followed by TY-fluorophore.

I. tissue preparation and hybridization (day 1)

1. fix slides in 4% paraformaldehyde for 10 minutes at RT
2. wash slides 3 times with PBS, 3 minutes per wash
3. Proteinase K treat, 1μg/ml in 50mM Tris-Cl pH 7.5, 6mM EDTA
4. acetylate for 10 minutes at room temperature (5.3 ml triethanolamine, 0.525mL glacial HCl, 0.75 ml acetic anhydride in 400 ml final volume).
5. wash slides 3 times with PBS, 5 minutes per wash
6. pre-hybridization in 500 λ hybridization solution for >1 hour at RT
7. replace pre-hyb with 75 λ hybridization solution with probe, hyb overnight at 72°C. Probes should be a mixture of FITC and DIG labeled cRNA probes.

II. washes and antibody staining (day 2)

1. wash in 5X SSC pre-heated to 72°C
2. wash 2 times (30 minutes per wash) in 0.2X SSC
3. equilibrate slides in 0.2X SSC for 5 minutes at RT

4. equilibrate slides in buffer B1 for 5 minutes at RT

5. block with TNB blocking buffer (NEN) (0.1M Tris 7.5, 0.15M NaCl, 0.5% blocking reagent)

6. remove block, incubate slides with antibody in TNB buffer overnight at 4°C; use sheep anti-FITC-POD (Roche) 1:500.

III. detection (day 3)

make vectastain elite (Vector ABC kit) before starting following steps

1. wash 3 times with TNT (0.1M Tris 7.5, 0.15M NaCl, 0.05% Tween20) to make 100 ml, add 10 ml 1M Tris 7.5, 3 ml 5M NaCl, 250 μl 20% Tween20

2. add biotinyl tyramide 1:50 in amplification diluent for 5 minutes at RT (100 μl per slide). Mix on slide, by gently rocking during all tyramide incubations.

3. wash 3 times with TNT (5mins each).

4. add vectastain-elite (ABC kit) in TNT for 30 minutes at RT - add exactly 2 drops of REAGENT A to 5 ml of TNT, then add exactly two drops of REAGENT B, allow ABC reagent to stand for about 30 minutes before use.

5. wash 3 times with TNT (5mins each).

6. add fluorescein tyramide (FITC) 1:50 in amplification diluent for 5-10 minutes at RT

7. wash 5 times with TNT inactivate deposited HRP using a 10mM HCl treatment for 30-60 min at 20°C. Also if biotin or streptavidin excess is detected, can use a avidin/biotin block (Vector).

8. Incubate slides in antibody in TNB buffer overnight at 4°C; use sheep anti-DIG-POD (Roche) 1:500. (Can also use antibody incubations at 20°C for 3-4 hrs).

9. Repeat steps III 1 through 5.
10. Detect DIG probe using CY3 Tyramide 1:50 in amplification diluent for 5-10 minutes at RT.

11. Wash 5 times TNT

12. Coverslip with vectashield.