3. The membrane is then incubated at room temperature for 30 min with antibody-conjugated solution.
4. To eliminate the excess of unbound antibody, the membrane is incubated in washing buffer 2 × 15 min and then equilibrated for 3 min with buffer 3. Optical or chemiluminescent detection methods can be developed, depending on the type of substrate used.

3.3.1. Colorimetric Detection

1. The membrane is incubated in a plastic bag in the dark, without shaking, with a freshly prepared solution containing NBT (0.3375 mg/mL) and BCIP (0.175 mg/mL), in buffer 3 in a final volume of approx 5 mL/100 cm^2 of membrane.
2. Remove the substrate solution when a purple color appears (15 min–12 h). The color reaction is stopped by washing the membrane in TE buffer. The results can be documented by photocopying or by photography.

3.3.2. Chemiluminescent Detection

1. The chemiluminescent substrate CSPD® (10 mg/mL) is diluted 1:100 in buffer 3. The membrane is incubated for 5 min with 5 mL of the above freshly prepared solution/100 cm^2 in a plastic bag in the dark.
2. Cut the plastic bag at the top and remove the substrate solution (it can be re-used several times). Using forceps, slide the membrane up the sides of the bag so that any excess liquid remains in the bag (do not allow the membrane to dry). The membrane is then sealed damp in a new plastic bag and incubated for 15 min at 37°C. Make a first exposure to X-ray film at room temperature for approx 15 min. Additional exposures may be taken, since luminescence continues for at least 24 h. Do not let the membrane dry if reprobing is intended (*see* **Note 6**). Store the membranes at 4°C in 2X SSC.

4. Notes

1. An easy way to double-check that the digoxigenin has been incorporated into the transcript RNA is to compare the electrophoretic mobility in TBE-agarose gels of the transcription products obtained in the presence and absence of the precursor DIG-UTP. If the digoxigenin has been incorporated into the cRNA, its electrophoretic mobility will be slower than that of the unlabeled transcript. Alternatively, transcription products can be serially diluted and spotted on nylon membranes, which can be developed as described in the general method.
2. In some cases, no or very low yields of digoxigenin-labeled transcripts are obtained. A dose-dependent inhibition of SP6, T7, and T3 RNA polymerases by the DIG-UTP precursor has been previously described ***(11)***. In addition, some templates are not suitable for the transcription reaction. In this situation, the manufacturer of DIG-UTP recommends recloning the cDNA, so that another RNA polymerase can be used. However, Heer et al. ***(11)*** have shown that lowering the relative amount of DIG-UTP could circumvent this problem. In our hands, templates having a size in the range of 1–2.7 kb gave the best yields when using