tives, or, alternatively, the presence of glycoproteins that bind avidin- or biotin-binding proteins can give unworkably high background. Another widely used molecule to nonradioactively label nucleic acids is the hapten digoxigenin, which is bound via a spacer arm (11 carbon residues) to uridin-nucleotides and is incorporated enzymatically into nucleic acids by standard methods. The protocol described here is based on probes labeled with digoxigenin. The fact that methods based on the biotin–avidin complex have not been included in this chapter does not mean that they are less efficient, but only that the authors are less familiar with them. The reader is referred to excellent reviews and original papers for details on the biotin–avidin system ***(5–9)***.

The following procedure is divided into three steps. The first involves the synthesis of the labeled probe by incorporating the digoxigenin into a cRNA by means of an in vitro transcription reaction, or into DNA by the random-priming method. The second part describes the preparation of the samples and the molecular hybridization reaction with the appropriate probe. After hybridization and blocking steps, bound probes are detected by high-affinity antibody Fab-fragments coupled to alkaline phosphatase. The third part implies the development of the detection method. Although chemiluminescent detection methods have been reported to be much more sensitive than colorimetric methods, the latter are also described, because of their convenience for testing field samples in less well equipped laboratories.

2. Materials

2.1. cRNA Probes

1. 10X Transcription buffer: 400 m*M* Tris-HCl, pH 8.0 (20°C), 60 m*M* $MgCl_2$, 100 m*M* dithiothreitol (DTT), 20 m*M* spermidine, 100 m*M* NaCl (Boehringer Mannheim, Mannheim, Germany). Store at –20°C.
2. 10X Digoxigenin-RNA (DIG-RNA) labeling mixture: 10 m*M* ATP, 10 m*M* CTP, 10 m*M* GTP, 6.5 m*M* UTP, 3.5 m*M* Dig-UTP, pH 7.5 (20°C) (Boehringer Mannheim). Store at –20°C.
3. RNase inhibitor (40 U/μL) (Promega, Biotec, Madison, WI). Store at –20°C.
4. RNA polymerase T7, T3, or SP6 (Boehringer Mannheim) (40 U/μL). Store at –20°C.
5. DNase I, RNase-free (20 U/μL) (Promega). Store at –20°C.
6. 0.2*M* EDTA, pH 8.0. Store at room temperature.
7. TE buffer: 10 m*M* Tris-HCl, pH 8.0, 1 m*M* EDTA. Store at room temperature.
8. 4*M* LiCl. Store at 4°C.

2.2. DNA Probes

1. 10X Hexanucleotide mixture: 0.5*M* Tris-HCl, pH 7.2 (20°C), 0.1*M* $MgCl_2$, 1 m*M* dithioerythritol (DTE), 2 mg/mL bovine serum albumin (BSA), hexanucleotides 62.5 A_{260} U/mL, pH 7.2 (20°C) (Boehringer Mannheim). Store at –20°C.