2. 10X DIG-DNA labeling mixture: 1 m*M* dATP, 1 m*M* dCTP, 0.65 m*M* dTTP, 0.35 m*M* Dig-dUTP, pH 7.5 (20°C) (Boehringer Mannheim). Store at –20°C.
3. Klenow enzyme (2 U/μL) (Boehringer Mannheim). Store at –20°C.
4. 4*M* LiCl. Store at 4°C.

### 2.3. Dot-Blot Hybridization

1. Mortars.
2. 50 m*M* Sodium citrate, pH 8.5. Autoclaved. Store at room temperature.
3. Nylon membrane, positively charged (Boehringer Mannheim).
4. 20X SSC: 3*M* NaCl, 0.3*M* sodium citrate, pH 7.0. Autoclaved. Store at room temperature.
5. Blocking stock solution: 10% (w/v) blocking reagent (Boehringer Mannheim) in buffer 1 (*see* **Subheading 2.4., item 1**); autoclaved and stored at 4°C.
6. Prehybridization solution: 50% (v/v) deionized formamide, 5X SSC, 2% (w/v) blocking reagent (added from 10% blocking stock solution), 0.1% (w/v) *N*-lauroylsarcosine, 0.02% (w/v) sodium dodecyl sulfate (SDS). Store at –20°C.
7. Washing solution I: 2X SSC, 0.1% (w/v) SDS.
8. Washing solution II: 0.1% SSC, 0.1% (w/v) SDS.

### 2.4. Immunological Detection

1. Buffer 1: 0.1*M* maleic acid, 0.15*M* NaCl, pH 7.5 (20°C), adjusted with concentrated NaOH, autoclaved.
2. Washing buffer: 0.3% Tween-20 (Sigma, St. Louis, MO) in buffer 1.
3. Buffer 2: 1% blocking reagent (added from 10% sterile stock solution) in buffer 1.
4. Buffer 3: 0.1*M* Tris-HCl, pH 9.5, 0.1*M* NaCl, 50 m*M* $MgCl_2$. Discard the solution if a precipitate appears after a long storage period.
5. Antibody-conjugated solution freshly prepared: Dilute 1:10,000 the sheep antidigoxigenin Fab-fragments conjugated to alkaline phosphatase (750 U/mL) (Boehringer Mannheim), in buffer 2. Diluted solution is stable only for 12 h at 4°C. Undiluted preparation are stable at 4°C. Do not freeze.
6. 75 mg/mL Nitroblue tetrazolium (NBT), in 70% (v/v) dimethylformamide, and 50 mg/mL 5-bromo-4-chloro-3-indolyl phosphate (BCIP), in dimethylformamide (Boehringer Mannheim). Store at –20°C.
7. CSPD® substrate (10 mg/mL) (Tropix, Bedford, MA). Store at 4°C and protected from light.
8. X-Ray films.

## 3. Method

### 3.1. Synthesis of the Probe

The two methods described here for preparing the nonisotopic label require that part of the viral sequence is cloned into the polylinker site of a plasmid vector that contains a promoter for SP6, T7, or T3 RNA polymerase. For RNA probes, the plasmid vector must be digested with an appropriate restriction