

3. Microcentrifuge tubes: 2, 1.5, and 0.2–0.5 mL, to fit PCR machine.
4. Micropipetors, adjustable volume from 0.1 to 20 μL , 20 to 200 μL , and 200 to 1000 μL . It is wise to use a separate set solely for pipeting RT and PCR reagents, to reduce chances of crosscontamination.
5. Pipet tips for micropipetors. Filter tips further reduce the chance of crosscontamination for pipeting RNA and DNA extracts.

2.2. Solutions and Reagents

For detection of RNA viruses, considerable care must be taken to limit exposure of extracted RNA to active RNases. Materials and solutions should be treated as described in detail in Sambrook et al. (1), which can be summarized as follows: bake tools and containers at 180°C for >2 h (if heat-resistant); wearing protective clothing, treat autoclavable containers and solutions with 0.1% (v/v) diethyl pyrocarbonate (DEPC) overnight in fume hood, followed by autoclaving; because RNases cannot be removed from Tris-containing buffers with DEPC, prepare Tris-solutions from RNase-free Tris with DEPC-treated sterilized water and glassware; disposable plasticware (gloves, microcentrifuge tubes, tips, and so on) is usually RNase-free when supplied by manufacturers, and hence need not be treated, but should at all times be handled wearing clean gloves.

1. Acid-phenol:chloroform (5:1 [v/v]): Made from stocks or bought commercially. Store in a dark bottle at 4°C.
2. Chloroform: **Caution**—Highly toxic, avoid inhalation. Wear protective clothing, and only carry out manipulations in a fume hood. Store in the dark at room temperature.
3. Chloroform:isoamyl alcohol (24:1 [v/v]). Toxic: *See* chloroform.
4. CTAB extraction buffer: Dissolve 0.4 g cetyltrimethylammonium bromide (CTAB) in 2 mL 1M Tris-HCl, pH 8.0, 0.8 mL 0.5M EDTA, pH 8.0, 5.6 mL 5M NaCl, 5 mL sterile ddH₂O. Make up to 20 mL with sterile ddH₂O, autoclave at 10 psi for 15 min, and store at room temperature.
5. Distilled deionized water (ddH₂O).
6. Diethylpyrocarbonate (DEPC). **Caution:** Because this is a mutagen, wear gloves and treat solutions and glass/metalware in a fume hood.
7. DNA markers: 100 bp ladder (1 $\mu\text{g}/10 \mu\text{L}$). Mix the following: 190 μL sterile dH₂O, 40 μL 6X orange G loading dye, and 10 μL 100 bp ladder stock (1 $\mu\text{g}/\mu\text{L}$, e.g., Pharmacia 27-4001-01). Store in refrigerator or freezer.
8. DNA polymerase 5 U/ μL ; e.g., *Taq* polymerase (Promega M1865). Store at –20°C.
9. dNTP mix: 10 mM: 10 mM of dATP, dCTP, dGTP, and dTTP in sterile ddH₂O. Store at –20°C.
10. dNTP stocks: 100 mM deoxynucleotide triphosphates stocks (HPLC grade). Commercially available.