3.2. RNA Extraction from Virus Particles

1. Add 1 vol of RNA extraction buffer and 2 vol water-saturated phenol to virus solution, vortex for 30–60 s, and centrifuge at maximum speed in microcentrifuge for 5 min at room temperature.
2. Transfer the aqueous phase (upper) to a fresh tube, extract once with equal volumes of phenol:chloroform, and centrifuge for 5 min.
3. Extract the aqueous phase again with chloroform, and centrifuge for 2 min.
4. Transfer the aqueous phase to a fresh tube, add 2.5 vol of cold ethanol and 0.10 vol of 3*M* sodium acetate, pH 5.5, mix well, and centrifuge for 10 min at 4°C in a microcentrifuge.
5. Wash the RNA pellet with 70% cold (–20°C) ethanol, dry, and resuspend the RNA in ice-cold sterile water. Store at –70°C for long periods.

3.3. Total RNA Extraction from Leaf Tissue

1. Precool mortar and pestle on ice for several minutes.
2. Rapidly homogenize 50–100 mg infected leaf tissue in ice-cold mortar with pestle, and immediately add 600 μL RNA extraction buffer.
3. Rapidly transfer the mix to a microtube containing an equal volume of phenol, vortex for 30–60 s and centrifuge at maximum speed in microcentrifuge for 5 min at room temperature.
4. Proceed as above from **step 2**.

3.4. Total RNA Extraction from Protoplasts

Total RNA can be extracted in a similar way from protoplasts: Discard the incubation medium and disrupt the protoplasts (approx $1–2 \times 10^6$) in 600 μL RNA extraction buffer, add an equal volume of phenol, and proceed following the same protocol described above.

4. Notes

1. *N. benthamiana* or *N. clevelandii* plants, normally used to propagate tombusviruses, can be inoculated with infected plant sap, purified virus or viral RNA. The protocol described in **Subheading 3.1.** is applicable to all tombusviruses and gives consistently reproducible results.
2. To obtain good virus yield, it is important to used a high speed homogenizer. The homogenization buffer must be prepare fresh. The low pH is important for the stability of virus particles; extraction media at pHs above neutrality are detrimental to tombusvirus particles.
3. The clarified sap (after **Subheading 3.1., step 4**) must be pale yellow. If plants are harvested too old and/or necrotic, the virus pellet may result in contamination by some dark material and may be difficult to clean further.
4. It is important to resuspend the virus pellet completely, especially after PEG precipitation, otherwise, much can be lost.