3.2. RNA Extraction

1. Vortex the virus pellets in buffer C, and add an equal volume of VEBA. Immediately pour the mixture into a tube containing an equal volume of phenol:chloroform. For total extraction volumes of 20 mL or less, a 50-mL disposable tube may be used with a wrist-action shaker. For larger volumes, use a beaker or flask.
2. Shake or stir the extraction for 15 min at room temperature.
3. Centrifuge the emulsion at top speed in a tabletop centrifuge for 10 min.
4. Remove the aqueous phase to a fresh tube containing the same volume of phenol:chloroform. Also, remove most of the fluffy interface. Repeat **steps 2** and **3**.
5. Remove only the aqueous phase to a fresh tube containing phenol:chloroform, and repeat **steps 2** and **3** above.
6. Remove only the aqueous phase to a clean 30 mL Corex tube, and add 2–2.5 vol of absolute ethanol. Store at –20°C for several hours or overnight.
7. Centrifuge the ethanol precipitate at 12,000*g* at 4°C for 30 min.
8. Pour off the supernatant and dry the pellets under vacuum.
9. Resuspend the pellets in 5 mL of NAE per tube, with vortexing. If the RNA pellet is very large, freezing and thawing can help resuspend the pellet. Concentrations of RNA can be as high as 10 mg/mL before saturation in water.
10. Add 12.5 mL absolute ethanol and store at –20°C as in **step 6**. Repeat **steps 7** and **8**.
11. Resuspend the pellets in 0.5 mL of NAE, and transfer to an Eppendorf tube. Add 1 mL of absolute ethanol for a final precipitation.
12. Centrifuge at top speed in a microcentrifuge for 10 min. Pour off supernatant, dry pellets under vacuum, and resuspend in 0.5 mL of 0.1 m*M* EDTA. Dilute 10 µL into 1 mL of water, and measure the OD at 260 and 280 nm. The 260/280 ratio should be 2. The extinction coefficient for RNA is 25 ***(23)***. The virus is about 18% RNA. Store the RNA at –20°C.

4. Notes

1. Alternate buffers for PSV are: buffer A, 0.1*M* sodium citrate, pH 7.0, 20 m*M* EDTA, 0.1% thioglycolic acid (v/v, add just before use); extraction is done with 2 mL buffer A and 2 mL chloroform per gram of tissue; initial pellets are resuspended in water, and 0.1 vol of CMV buffer A, plus Triton X-100 to 2% is added before stirring; the second pellet is resuspended in water, and 0.1 vol of CMV buffer A is added for storage; cushion I, 0.1*M* sodium citrate, pH 7.0, 1 m*M* EDTA, 10% sucrose; cushion II, 50 m*M* sodium citrate, pH 7.0, 0.5 m*M* EDTA, 10% sucrose.
2. Alternate buffers for TAV are: buffer A, 0.1*M* sodium or potassium phosphate, pH 7.0, 0.5% thioglycolic acid; buffer B, 20 m*M* phosphate, pH 7.0, 1% Triton X-100, buffer C, 20 m*M* phosphate, pH 7.0, or, if viral RNA is to be extracted, virus may be resuspended in water (avoid EDTA in the final resuspension buffer); cushion I, 0.1*M* phosphate, pH 7.0, 10% sucrose; cushion II, 20 m*M* phosphate, pH 7.0, 10% sucrose.