

3. Buffer C: 10 mM Tris-HCl, 5 mM MgCl₂, pH 7.8 (autoclaved).
4. 10% Sucrose: 10 g of sucrose dissolved in 100 mL of buffer C.
5. 40% Sucrose: 40 g of sucrose dissolved in 100 mL of buffer C.

The last two solutions are used to prepare sucrose gradients and should be kept aliquoted and frozen to avoid bacterial contamination.

2.2. Viral RNA Extraction

1. Proteinase K (20 mg/mL in water) (Sigma).
2. 20% SDS (w/v) (Bioprobe).
3. Phenol saturated with 50 mM Tris-HCl, pH 8.0 (Bioprobe).
4. Chloroform/isoamyl alcohol (24/1, v/v) (Prolabo).
5. 3M Sodium acetate, pH 5.3 (autoclaved) (Sigma).
6. 96% Ethanol (kept at -20°C) (Carlo Erba).
7. TE: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 (autoclaved).

2.3. Gel Electrophoresis of RNA

1. 10X MOPS/EDTA, pH 7.0: 500 mM MOPS (Sigma), 10 mM EDTA, adjust to pH 7.0 with NaOH (Sigma) (autoclaving of this buffer will result in a yellow color that does not interfere with the migration).
2. Buffer D: 294 µL 10X MOPS/EDTA, pH 7.0, 706 µL H₂O.
3. Buffer E: 89 µL formaldehyde (Sigma) (37%, 12.3M), 250 µL formamide (Sigma) (freshly deionized).
4. Dyes mix: 322 µL buffer D, 5 mg xylene cyanol (Serva), 5 mg bromocresol green (Sigma), and 400 mg sucrose.
5. Gel-loading buffer: 2 µL formaldehyde (37%, 12.3M), 5 µL formamide (freshly deionized), and 7 µL Dyes mix.
6. Electrophoresis buffer: 1X MOPS/EDTA, pH 7.0.
7. Gel preparation: The 0.8% agarose gel is prepared by dissolving 0.4 g agarose (Eurobio) in 36 mL of H₂O, cooling to 70°C, and adding 5 mL of 10X MOPS/EDTA, pH 7.0, and 9 mL 37% formaldehyde (final concentration 2.2M).
8. Gel-staining solution: 10 mM sodium acetate (Merck), 10 mM magnesium acetate (Merck), 0.05% (w/v) Ortho-toluidine blue (Sigma), pH adjusted to 5.5 with acetic acid.

3. Methods

The following procedure was adopted for purifying ACLSV. All steps are done at 4°C on a refrigerated bench.

3.1. Bentonite Suspension Preparation

The protocol we currently use in our laboratory is the one proposed by Lister and Hadidi (19), with a few modifications. For a typical batch, 10 g of bentonite (Bentonite powder, Fisher Scientific) are suspended in 200 mL of buffer A