Because of its general utility for studies of the replication of SYNV ***(16,17)*** and its potential use in studies of other viruses, we have introduced this method here.

3.2. Materials

Buffers: All solutions are prepared in baked glassware using analytical grade reagents, and are autoclaved before using. Use ultrapure ribonuclease free sucrose and maintain RNase-free reagents by wearing gloves when handling them, and by using flamed or baked spatulas and autoclaved stir bars when preparing solutions. Add EGTA, β-mercaptoethanol, and Triton X-100 to appropriate buffers, just before use.

1. EGTA stock: Add 38 g of EGTA to 85 mL of H_2O. Adjust pH to 8.0 with 5*M* NaOH and adjust the volume to 100 mL. Store at –20°C in 5-mL aliquots.
2. Triton X-100 (20%): Add 20 mL of reagent grade Triton X-100 to 80 mL of dd-H_2O. Autoclave, then mix occasionally, as the temperature cools, to prevent separation of the phases. Store at room temperature.
3. Extraction buffer: 200 m*M* Tris-HCl, pH 9.0, 400 m*M* KCl, 35 m*M* $MgCl_2$, 25 m*M* EGTA, and 200 m*M* sucrose and 1% Triton X-100. To prepare 2X extraction buffer, add 24.22 g Tris base, 29.82 g KCl, 7.10 g $MgCl_2$, and 68.46 g sucrose (RNase-free) to 450 mL of H_2O. Adjust the pH to 9.0 with HCl, and bring the volume to 500 mL. Store at –20°C indefinitely as 100-mL aliquots. Just before use, dilute the necessary volume of 2X extraction buffer with an equal volume of a dilution buffer containing 50 m*M* EGTA from the 1*M* stock solution, 2% β-mercaptoethanol, and 2% Triton X-100 diluted from the 20% stock (*see* **Subheading 3.4., Note 1**).
4. Sucrose pad buffer: 40 m*M* Tris-HCl, pH 9.0, 200 m*M* KCl, 35 m*M* $MgCl_2$, 5 m*M* EGTA, and 1.75*M* sucrose. Add 1.92 g Tris Base, 5.96 g KCl, 3.44 g $MgCl_2$ and 240 g sucrose to 450 mL of H_2O. Adjust the pH to 9.0 with HCl, and the volume to 500 mL. Store at –20°C in 50-mL aliquots. Add 250 µL of 1*M* EGTA to each 50-mL aliquot just before use.
5. Resuspension buffer: 40 m*M* Tris-HCl, pH 8.5, 200 m*M* KCl, 30 m*M* $MgCl_2$, and 5 m*M* EGTA. Add 0.48 g Tris base, 1.49 g KCl, and 2.03 g $MgCl_2$ to 95 mL of H_2O. Bring the pH to 8.5 with HCl. Store at –20°C in 12-mL aliquots. Add 60 µL of 1*M* EGTA (5 m*M*) just before use.
6. Sucrose gradient buffer: 40 m*M* Tris-HCl, pH 8.5, 20 m*M* KCl, and 10 m*M* $MgCl_2$. To make 10X stock sucrose gradient buffer, add 4.84 g Tris base, 1.49 g KCl, and 2.03 g of $MgCl_2$ to 95 mL of H_2O. Adjust the pH to 8.5 with HCl, and the volume to 100 mL. Store at –20°C in 5-mL aliquots.

3.3. Methods

3.3.1. Method for Polyribosome Extraction

1. Prechill all buffers, mortars, pestles, rotors, and centrifuges to 4°C (*see* **Subheading 3.4., Note 2**).