autoclave, and equilibrate the buffer to 4°C. Add Tris-HCl, pH 8.0, to 25 m*M* and β-mercaptoethanol to 10 m*M*. Add PMSF to 1 m*M* immediately before use and adjust the volume to 500 mL.

3. 95% Percoll stock solution: This solution contains 95% Percoll (Pharmacia, Uppsala, Sweden)/5% mannitol buffer. Prepare the 95% Percoll stock solution in a sterile beaker by mixing 70 mL of Percoll, 1.842 mL of 1*M* Tris-HCl, pH 8.0, 368 μL of 1*M* $MgCl_2$, and 3.37 g of mannitol. Remove 19.75 mL for the 75% Percoll stock solution. Just before use, add 57.5 μL β-mercaptoethanol and 216 μL of 200 m*M* PMSF stock solution. Do not autoclave or filter the Percoll containing buffers.
4. 75% Percoll stock solution: Make a 25-mL solution containing 75% Percoll/25% mannitol buffer. To prepare the 75% Percoll buffer, mix 19.75 mL of the 95% Percoll stock solution from **step 3** and dilute with 5.25 mL of mannitol buffer containing 10 m*M* β-mercaptoethanol and 1 m*M* PMSF added immediately before use.
5. Polymerase elution buffer: Prepare a 5-mL solution containing 40% v/v glycerol, 200 m*M* $(NH_4)_2SO_4$, 25 m*M* hydroxyethylpiperazine ethane sulfonic acid (HEPES, pH 8.0), 5 m*M* $MgCl_2$. Immediately before use, add dithiothreitol (DTT) to 3 m*M*, Aprotinin and Pepstatin to 1 μg/mL each, Leupeptin to 0.5 μg/mL, and PMSF to 1 m*M*.

4.3. Methods

4.3.1. Method for Isolation of SYNV Polymerase

1. Harvest 100 g of SYNV-infected *N. edwardsonii* leaf tissue displaying netting symptoms at 10–14 d postinoculation *(7)*. Tissue frozen at –80°C may be stored for several months without detectable loss in polymerase activity.
2. Grind tissue in liquid nitrogen in a stainless steel blender. Using a reostat, start the blender at medium speed, and add some liquid nitrogen. Then add the frozen tissue, hold the cover top down with an asbestos glove, and grind the tissue at the highest setting in three 30-s bursts. Lower the speed to a medium setting, and remove the top, letting the remaining nitrogen evaporate. Do not stop the blender between the bursts or until the nitrogen evaporates, because it will freeze up and you will not be able to start it again.
3. Quickly transfer the ground tissue into an Omnimixer (Dupont, Wilmington, DE). Add 400 mL of chilled nuclei extraction buffer, and mix at the highest setting to get a uniform slurry. **Step 3** and all subsequent isolation steps are performed at 4°C.
4. Put the slurry in a 600-mL beaker containing a stirbar. While the solution stirs, add Nonidet P-40 (NP40) to 0.6%, and stir for 5 min.
5. Pour the cold slurry through autoclaved 350-μm, 62-μm, then 44-μm nylon mesh filters. This is accomplished by attaching the meshes to a 2-L plastic beaker with rubber bands. First, put a course mesh net on the bottom, then add the fine mesh filter, the middle mesh, and another course mesh net on the top.
6. Filter the slurry by gravity flow. Then remove one mesh at a time and squeeze the remaining liquid into the beaker. Save this filtrate. Next, scrape the material from