6. Layer the supernatant on a sucrose pad consisting of 5–8 mL buffer D (depending on tube size) and centrifuge 90 min at 100,000*g* at 4°C. Resuspend the pellets in buffer A and centrifuge at 8000*g* at 4°C for 10 min.
7. Layer aliquots of the supernatant on 10–40% sucrose density gradients (buffer E) and centrifuge for 2 h at 100,000*g*. Collect the high A_{254} absorbing fraction (*see* **Note 17**).
8. Dialyze the virus-containing fractions overnight against several changes of buffer A, or simply dilute the fractions and centrifuge at high speed to concentrate the virus.

3.3. Purification of Legume-Infecting Potyviruses

This procedure has been used successfully by several laboratories for purification of strains of bean common mosaic virus, bean common mosaic necrosis virus, and bean yellow mosaic virus. It should be applicable to many other related legume-infecting viruses. The method presented here is a modification of the procedure of Morales, as cited in **ref. *4***.

1. Harvest 200 g leaf tissue 10–11 d after inoculation and homogenize with 200 mL cold grinding buffer (buffer B). Add, for each 200 mL buffer, 50 mL chloroform and 50 mL carbon tetrachloride and homogenize briefly (about 10 s).
2. Centrifuge for 5 min at 5000*g*. Pour off supernatant carefully (*see* **Note 18**).
3. Add PEG to 6% (w/v), stir 1 h at 4°C, and centrifuge for 10 min at 12,000*g* (*see* **Note 19**).
4. Allow pellet to resuspend undisturbed in buffer A for at least 6 h, and then clarify by centrifugation for 10 min at 12,000*g* (*see* **Note 20**).
5. Add 2 mL of a 20% (w/v) PEG solution in 0.02*M* Tris-HCl, pH 8.2, per 5 mL virus suspension. Incubate 1 h at 4°C.
6. Centrifuge for 10 min at 17,000*g*.
7. Resuspend pellets in buffer C. You can resuspend this pellet overnight at 4°C.
8. Centrifuge for 10 min at 12,000*g*.
9. Add CsCl to 35% (w/v), gently dissolve, and centrifuge for 18 h at 120,000*g* (*see* **Note 21**).
10. Recover virus zone. Dilute the CsCl with buffer C and concentrate by centrifugation at 84,500*g* for 90 min at 4°C.

3.4. Purification of Rymoviruses

This method was originally developed by Brakke and Ball ***(5)*** and subsequently modified by Sherwood ***(6)***. Although this procedure works quite well, it has the disadvantage that relatively large volumes of extract must be subjected to ultracentrifugation because a PEG precipitation step is not (and cannot be) used. Thus, a large capacity ultracentrifuge rotor is very useful here.

1. Grind young leaves (about 3 wk after inoculation) in 2 mL buffer A per gram leaf tissue. Squeeze tissue through cheesecloth as described above (*see* **Notes 22** and **23**).
2. Check pH of filtrate. If above pH 6.1, lower to pH 6.1 with acetic acid.