4. Notes

1. Best results are frequently obtained from protein extracts prepared from younger plants (before flowering), rather than from older plants. Root material is sometimes preferable because of the lower levels of chlorophyll pigments that can affect detection, especially of lower mol-wt (<14 kDa) proteins.
2. We crush plant material in plastic bags (75 × 120 mm, 100 μ*M* thickness) using a roller. We then add buffer and continue to extract the sample with the roller for a further 30 s. This method is very rapid and there is little chance of contamination between samples.
3. An alternative sample preparation involves the use of magnetic beads. We have used Dynabeads M-280 (tosyl activated) (Dynal A.S., cat. no. 142.03) to covalently attach affinity purified antibodies. Use of these beads, in our hands, has resulted in a 20-fold improvement in sensitivity for the detection of white clover mosaic potexvirus coat protein (CP), compared to the use of the protocol outlined in this chapter. We have also shown that nonstructural gene products of viruses overexpressed in a baculovirus system can be affinity-purified using antibodies attached covalently to the beads. The bead attachment procedure is described by the supplier. A simple protocol for its use is as follows. Leaf tissue is crushed in a plastic bag (*see* **Note 2**) using 2 vol (2 mL/g tissue) of bead extraction buffer. The extract is transferred to a 1.5-mL Eppendorf tube and clarified by centrifugation for 5 min in a microcentrifuge. The supernatant (1 mL) is tumbled end-over-end for 2 h with 10–20 μL (100–200 μg) of antibody-conjugated beads. With the use of a Dynal MPC concentrator, the magnetic beads are washed once with PBS-T, and the affinity-purified protein is removed from the antibody by boiling in the presence of 25 μL 1X SDS-PAGE sample buffer for 2 min. The sample is centrifuged for 2 min and the supernatant recovered and analyzed by SDS-PAGE and Western blotting, as outlined in this chapter.
4. Western blotting of potyviral CP transgene products can be achieved using the antipotyvirus group IgG (AGDIA, Elkhart, IN; cat. no. F272). We use the following modifications to the procedure (Y. Y. Wang, personal communication). The extraction should be performed with 10 vol (10 mL/g tissue) of 2X running buffer (6.04 g Tris-HCl, pH 8.3, 28.8 g glycine, 2 g [w/v] SDS; make up to 1 L). Samples are clarified following extraction by centrifugation for 10 min in a microcentrifuge. The supernatant (80 μL) is mixed thoroughly with 5X SDS-PAGE (20 μL) sample buffer. The extract is boiled for 3 min and clarified by centrifugation in a microcentrifuge to remove insoluble material before SDS-PAGE. Transfer to nitrocellulose is as described in **Subheading 3., step 2**. Following transfer, the membrane is rinsed in PBS and dried at room temperature. Substitute the block and antibody dilution solutions for: 1X PBS; 5% (w/v) nonfat milk powder; 2% (w/v) BSA. The membrane is incubated with AGDIA antipotyvirus group IgG at 1:100. A secondary antibody labeled with horseradish peroxidase is used, in combination with chemiluminesence (ECL, Amersham, Little Chalfont, UK), to detect the potyvirus CP transgene.