1. Add 9 mL distilled water to 1 mL of crude antiserum.
2. Slowly add 10 mL neutralized saturated ammonium sulfate (Sigma) while stirring (*see* **Note 11**).
3. Remove from stirring and leave to precipitate for about 1 h at room temperature. The resulting solution should appear viscous and cloudy.
4. Centrifuge for 15 min at 9000*g* and retain precipitate.
5. Dissolve the precipitate in 2 mL of half-strength PBS.
6. Add the dissolved precipitate to a Centriprep tube, top up to the mark with half-strength PBS and spin at 9000*g* (speed not critical) for 20 min. Discard the liquid that has drained into the central part of the tube. Replace with half-strength PBS up to the mark and repeat twice more, to remove traces of ammonium sulfate.
7. Measure the optical density at a wavelength of 280 nm (*see* **Note 12**) and adjust by dilution in half-strength PBS until the reading is 1.4 (this dilutes the γ globulin to 1 mg/mL). The solution can be concentrated by further spinning in the Centriprep tube, if the reading is <1.4.
8. Aliquot to volumes suitable for storage (1 mL is often convenient) and store at –20°C, or lyophilized. Sodium azide (Sigma) may be added to 0.02% w/v as a preservative (*see* **Note 13**).

3.4. Preparation of Antibody–Enzyme Conjugate (Using Alkaline Phosphatase Suspension in Ammonium Sulfate)

1. Centrifuge suspension containing 5000 U of enzyme to precipitate (3 min at high speed in a microcentrifuge).
2. Dissolve precipitate in 2 mL purified γ-globulin (1 mg/mL, prepared as above).
3. Add to a Centricon tube and centrifuge at 9000*g* for 20 min (*see* **Note 14**). Replace with half-strength PBS up to the 2-mL mark, and repeat twice more, to remove traces of ammonium sulfate.
4. Add fresh gluteraldehyde (Sigma) to 0.05% (it may be convenient to prepare a 5% gluteraldehyde solution in distilled water and add 20 µL to the 2 mL of antibody solution). Mix well.
5. Leave for 4 h at room temperature or overnight at 4°C. A faint brown color may develop.
6. Repeat **step 3** to remove traces of gluteraldehyde. Final volume should be 2 mL.
7. Add bovine serum albumin (Sigma) (BSA) to 5 mg/mL w/v, mix to dissolve, and store at 4°C.
8. Sodium azide may be added to 0.02% w/v, to enhance storage life (*see* **Note 13**).

3.5. Screening and Evaluation of Antibodies

Before using antibodies in an assay, their efficacy should be tested against the virus or virus group concerned, and working dilutions optimized. They should be screened against large panels of virus isolates and a range of cultivars of healthy plants. It is important to include closely related viruses, to ensure that crossreactivity does not occur. In the case of MAbs, early screening