6. Conjugate buffer: Sample buffer with the addition of 2 g/L ovalbumin (Sigma, Grade V).
7. Substrate buffer: 1*M* diethanolamine adjusted to pH 9.8 with 2*N* HCl. Buffers should be kept refrigerated. An antimicrobial agent, e.g., 0.2 g/L of NaN_3, should be added to coating, sample, and conjugate buffers if prolonged storage is anticipated. (**Caution:** NaN_3 is highly toxic.)
8. Wash buffer: PBS containing 0.05% Tween-20 (PBS-Tween). Wash buffer can be stored as a 10X concentrate without preservative and used freshly diluted.
9. Substrate: *p*-nitrophenyl phosphate (1 mg/mL) is the substrate normally used with alkaline phosphatase. Powder or tablets work equally well.
10. Purified viral protein or virions, concentrated at least to 0.1 μg/mL.

3. Method

Since the aim is to compare concentrations of viral protein in many samples of tissues, perhaps assayed over substantial periods of time, standardization and faithful replication of procedures at all stages from growth of plants to the completion of ELISA are required to maximize precision in resultant data.

3.1. Collection of Leaf Tissue and Extraction of Sap

1. Either a disk of tissue from the lamina of a leaf or an entire leaflet or leaf can be the standard unit of tissue for assay. If sampling spans days or weeks, tissue should be collected at the same time each day from plants maintained under uniform conditions of photo period, temperature, nutrition, and hydration.

   It is advisable to refrigerate sampled tissue immediately after collection if the interval before sap extraction will exceed a few minutes.
2. Sap may be extracted mechanically (in which case, it is necessary to know the dry matter content of the tissue) and aliquots added to sample buffer predispensed into microtiter wells, or tissue can be macerated in a proportionate volume of sample buffer before transfer of aliquots to microtiter wells. Either way, the interval from sap extraction to placement in wells should be minimized.

3.2. ELISA Procedure

1. Add trapping antibody diluted appropriately in coating buffer. Normally, a 1 μg/mL IgG concentration is used. Any volume between 50 and 200 μL per well can be chosen. It should, though, be equal to or slightly greater than the volumes used in subsequent stages of the procedure. Incubate at 4°C overnight.
2. Mechanically or manually empty trapping antibody solution from the wells and wash three times with PBS-Tween (*see* **Note 1**). Washed plates should be inverted and tapped onto tissue paper to dislodge droplets remaining in wells. Plates can be used immediately for the next stage, or, alternatively, wrapped in polythene to prevent drying, and stored for days at 4°C or for up to 6 mo at –20°C.
3. If necessary, at this stage and/or after washing out of test antigen, plates can be treated to block any remaining nonspecific reactive sites on the polystyrene surfaces as a means of reducing high background-absorbance values (*see* **Note 2**).