

3. PBS-T: Add 0.5 mL Tween-20 to 1 L of PBS.
4. PBS-TPO: 2.0 g polyvinylpyrrolidone (mol wt 40,000), 0.2 g bovine serum albumin (BSA), make up to 100 mL with PBS-T. Make up fresh each day.
5. Chloroform:isoamyl alcohol (IAA): (24:1).
6. 1.25 mM dNTPs.
7. 1% Sodium dodecyl sulfate (SDS), 1 mM ethylenediamine tetra-acetic acid (EDTA), 10 mM Tris-HCl, pH 7.5.
8. 3M NaAc.

2.2. Chemicals

1. Protein A.
2. Mineral oil.
3. Absolute ethanol.
4. RNase inhibitor (RNasin, Promega, Southampton, UK).
5. Superscript reverse transcriptase + buffer (Gibco-BRL, Paisley, UK).
6. *Taq* polymerase + buffer.
7. Forward and reverse primers (*see Note 4*).

2.3. Equipment

1. Microcentrifuge.
2. Water baths.
3. Micropipets.
4. -20°C Freezer.
5. Microtiter plates (*see Note 2*).
6. PCR machine.

3. Methods

3.1. Immune Capture (*see Note 1*)

1. Coat microtiter plates with 2 µg/mL protein A in coating buffer for 3 h at 37°C, using 200 µL per well. Wash plates three times for 30 s in PBS-T.
2. Add antibody (*see Note 3*) diluted in PBS-TPO, using 200 µL per well, and incubate for 1 h at 37°C. Wash plates three times for 30 s in PBS-T.
3. Grind 0.5 g leaf tissue of superinfected plant and of control plants in 300 µL PBS-TPO.
4. Add virus or plant samples to well, diluted if necessary in PBS-TPO, using 150 µL per well, and incubate overnight at 4°C. Wash plates three times for 30 s in PBS-T.
5. After final wash, extract viral RNA from plate with 1% SDS, 1 mM EDTA in 10 mM Tris-HCl, pH 7.5. Use 200 µL per well and incubate at 50°C for 5 min.
6. Immediately transfer the samples to Eppendorf tubes containing 100 µL each of phenol and chloroform:IAA. Vortex for 5 min.
7. Centrifuge at 12,000g in a microcentrifuge for 15 min and recover the aqueous phase.