ELISA protocol for TMV detection

1. Plan the layout of the ELISA plate ensuring that the necessary buffer, positive and negative controls are added. Always use duplicate wells.

2. Prepare samples (0.4 g) in labelled plastic bags. Avoid using stem or leaf vein material. Also label the eppendorf tubes to be used in step 3.

   To each bag add 800 μl of coating buffer. Grind the leaf material using a glass rod or similar smooth ended object ensuring that the plastic bag is not damaged. Make sure the leaf material is thoroughly homogenised. Maintain on ice or at 4°C.

   In the case of dried leaf material, use 0.1 g and up to 4 ml of extraction buffer.

3. Transfer the samples to clean, labelled eppendorf tubes. Maintain on ice or at 4°C.

4. Coat the plates with 100 μl of each sample. Incubate at 37°C for 1 hours.

   During the incubation time, prepare the PRSV antibody by diluting in PBS-T (1:2000 dilution). For one plate, use 5 μl of antibody in 10 ml of PBS-T.

5. Pour out the samples and wash 3 times with PBS-T using a wash bottle. Ensure that the wells are free from excess liquid but not totally dry.

6. Add 100 μl of diluted PRSV antibody to each well. If using more than one antibody, take care in aliquoting them. Incubate at 37°C for 1 hour.

   During the incubation time, prepare the secondary conjugated antibody by diluting in PBS-T (1:2000 dilution). For one plate, use 5 μl of antibody in 10 ml of PBS-T.

7. Wash as in step 5.

8. Add 100 μl of diluted antibody to each well. If using more than one antibody, take care in aliquoting them. Incubate at 37°C for 1 hour.

   During the incubation time, prepare the PNPP substrate by diluting 50 μl of PNPP stock in 10 ml of diethanolamine buffer. Store in the dark at 4°C until use.

9. Wash as in step 5.

10. Add 100 μl of PNPP to each well. Allow the colour to develop – in the case of PRSV this can be as quick as five or ten minutes but it can be left for some hours or even overnight as the antibody has little background. Read the plate by eye or using an ELISA plate reader (absorbance 405 nm). Stop the reaction by adding 50 μl of 3 M NaOH.

    The plates can be stored on top of a moist paper towel in a sealable plastic bag at 4°C.

Reagents

A. Coating buffer (0.05 M carbonate buffer, pH 9.6) 500 ml

Na_2CO_3	0.795 g
$NaHCO_3$	1.465 g
dH_20	480 ml

If need be, adjust the pH to 9.6 (although this should not normally be required) and make up to 500 ml. Autoclave and store at 4°C. If you don't autoclave the buffer, store for a maximum of 14 days at 4°C

B. 10X PBS, 1000 ml

NaCl	80 g
Na_2HPO_4	14.4 g
KH_2PO_4	2.4 g
KCl	2 g
dH_20	800 ml

After adding 800 mls dH_2O, ensure that reagents are re-suspended, pH to 7.2 before finally making up to 1000 ml with dH_2O . Autoclave and store at room temperature. After opening, store at 4°C.

C. PBS-T (1X)

10 X PBS	100 ml
Tween-20	500 µl
dH_20	900 ml

D. Diethanolamine buffer

Diethanolamine	48.5 ml
dH_20	400 ml

Adjust pH to 9.8 and make up to 500 ml. Wrap bottle in aluminium foil and store at 4°C. **DO NOT AUTOCLAVE.**

E. 3 M NaOH

NaOH	24 g
Make up to 200 ml with dH_20	

DO NOT AUTOCLAVE

F. Antibodies

(i) Goat anti-rabbit IgG (H+L) Alkaline Phosphatase Conjgate (ZYMED, Cat # 81-6122). Store at 4°C. Use at 1:2000 (or up to 1:10,000) dilution.

TMV:

G. Nunc maxi-sorb flat bottom ELISA plates
OR
EIA/RIA flat bottom, high binding plates (Costar, Cat# 3590)

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	B										
B	SAP	SAP										
C	+ve	+ve										
D												
E												
F												
G												
H												