

## **ELISA protocol for PRSV (and ZYMV) 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  $\mu$ l 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  $\mu$ l 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  $\mu$ l 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  $\mu$ l 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  $\mu$ l of antibody in 10 ml of PBS-T.

7. Wash as in step 5.

8. Add 100  $\mu$ l 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  $\mu$ l 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  $\mu$ l of PNPP to each well. Allow the colour to develop – in the case of PRSV this can be as quick as 5 or 10 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  $\mu$ l of 3 M NaOH.

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

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## **Reagents**

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

Na <sub>2</sub> CO <sub>3</sub>	0.795 g
NaHCO <sub>3</sub>	1.465 g
dH <sub>2</sub> O	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 <sub>2</sub> HPO <sub>4</sub>	14.4 g
KH <sub>2</sub> PO <sub>4</sub>	2.4 g
KCl	2 g
dH <sub>2</sub> O	800 ml

After adding 800 mls dH<sub>2</sub>O, ensure that reagents are re-suspended, pH to 7.2 before finally making up to 1000 ml with dH<sub>2</sub>O . 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 <sub>2</sub> O	900 ml

D. Diethanolamine buffer

Diethanolamine	48.5 ml
dH <sub>2</sub> O	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 <sub>2</sub> O	

**DO NOT AUTOCLAVE**

F. Antibodies

- (i) Goat anti-rabbit IgG (H+L) Alkaline Phosphatase Conjugate (ZYMED, Cat # 81-6122). Store at 4°C. Use at 1:2000 (or up to 1:10,000) dilution.
  
- (iv) PRSV: Polyclonal antibody from rabbit. This polyclonal antiserum is very robust and specific for PRSV, with some cross reactivity with ZYMV. It is stored in 50% glycerol with sodium azide. Store at -20°C. Use at 1:1000 for quick reactions or up to 1:5000.

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G. Nunc maxi-sorb flat bottom ELISA plates

OR

EIA/RIA flat bottom, high binding plates (Costar, Cat# 3590)

<b>H</b>	<b><u>1</u></b>	<b><u>2</u></b>	<b><u>3</u></b>	<b><u>4</u></b>	<b><u>5</u></b>	<b><u>6</u></b>	<b><u>7</u></b>	<b><u>8</u></b>	<b><u>9</u></b>	<b><u>10</u></b>	<b><u>11</u></b>	<b><u>12</u></b>
<b><u>A</u></b>	<b>B</b>	<b>B</b>										
<b><u>B</u></b>	<b>SAP</b>	<b>SAP</b>										
<b><u>C</u></b>	<b>+ve</b>	<b>+ve</b>										
<b><u>D</u></b>												
<b><u>E</u></b>												
<b><u>F</u></b>												
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