TECHNICAL SHEET No. 28

Virus detection: *Prunus necrotic ringspot virus*

Method: ELISA

General

Virus detected: *Prunus necrotic ringspot Ilarvirus* (PNRSV)
General method: ELISA

Developed by

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Goals

Identification of PNRSV strains and production of PNRSV ELISA Kits.

Introduction

Prunus necrotic ringspot virus

Phosphate Buffer Saline (PBS)	**Washing Buffer pH 7.4**	**Coating Buffer pH 9.6**
Distilled water 1.0	PBS 1.0 l	Distilled water 1.0 l
NaCl 8.0 g	TWEEN 20 0.5 ml	Na_2CO_3 1.6 g
$Na_2HPO_4.12H_2O$ 2.9 g		$NaHCO_3$ 3.0 g
KH_2PO_4 0.2 g		
KCl 0.2 g		

Conjugate Buffer pH 7.1-7.3	**Substrate Buffer pH 9.6 adjusted with concentrated HCl**
PBS TWEEN 100 ml	Distilled water 80.0 ml
PVP 2 g	Diethanolamine 9.7 ml
Ovalbumine 0.2 g	

Coating of ELISA plates

1. Dilute antibodies in coating buffer as recommended by the supplier.
2. Add 100 µl to each well, cover the plate.
3. Incubate for 3-4 hr at 37ºC.
4. Wash three times with PBS-TWEEN.

Antigen extraction and binding

1. Leaf tissues (1:10 w/v) were extracted in 0.1 M phosphate buffer Saline-Tween-Polyvinylpyrolidone (PBST-PVP), pH 7.4 containing 2% PVP, 0.05% Tween-20, 0.15 M NaCl, and 4 mM KCl.
2. Add 100 µl extracted sample/well; two wells were used per sample.
3. Incubate overnight at 4ºC.
4. Wash three times with PBS-TWEEN.

Conjugate

1. Dilute conjugated antibodies in conjugate buffer as recommended and add 100 µl to each well.
2. Incubate for 3-4 h at 37ºC.
3. Wash three times with PBS-TWEEN.

Color reaction

1. Dissolve 10 mg pNPP in 10 ml substrate buffer just before use.
2. Incubate for 1 h.
3. After incubation, the reaction was detected calorimetrically at A_{405} nm using an ELISA reader.
4. The test was considered positive when the mean absorbance value of a sample was over twice that of healthy controls.