TECHNICAL SHEET No. 3

Virus Detection: *Citrus tristesa virus* (CTV)

Method: DAS ELISA

General
Virus detected: Citrus Tristesa virus (CTV) from citrus green stems.
General method DAS ELISA

Developed by
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Goals
To develop a sensitive and easy method for CTV detection in infected stems.

Introduction

CTV is the most economically important virus problem affecting citrus worldwide and causes the death and decline of trees in sour orange rootstocks and a debilitating stem pitting disease in limes, grapefruit, and sweet oranges which affects trees on all rootstocks and reduces yield. CTV is today widespread in Israel, Morocco, India, China, Japan, Southern California, Florida, Argentina, Brazil, South Africa, Australia and southern Spain, and is moving into previously free, northern Spain. ELISA is the standard diagnostic method for CTV.

Materials and Methods

The anti-CTV antibody and the anti-CTV antibody alkaline phosphatase conjugate were from Bioreba

Coating

1. Dilute the CMV antibody 1:1000 in coating buffer. Coating buffer is for 1 liter (pH 9.6), in ddw: Na_2CO_3 1.59 g, $NaHCO_3$ 2.93 g, NaN_3 0.20 g.
2. Add 100- 200 µl to each well and cover plates tightly.
3. Incubate at 37°C for 4 hr or at 4-6 ^{o}C for 18 h.
4. Wash: Empty the wells and wash 3-4 times with washing buffer, remove any liquid by blotting the plate on paper to wells. Washing buffer is (for 1 liter, pH 7.4): in ddw, NaCl 8.00 g, KH_2PO_4 0.20 g, Na_2HPO_4 1.15 g, KCl 0.20 g, Tween20 0.50 g, NaN_3 0.20 g.

Antigen extraction and binding

1. Add 100-200 µl extraction buffer per well. Extraction buffer is: 20 mM Tris buffer (pH 7.4) containing 137 mM NaCl, 3 mM KCl, 2 % PVP 24kD, 0.05 % Tween 20 and 0.02 % NaN_3.
2. Cut the citrus green stem into small peaces (1 mm^2); add 1-2 pieces to the well containing the extraction buffer. Cover the plate tightly and incubate at 4-6 ^{o}C for 18 h.

3. Wash: Empty the wells and wash 3-4 times with washing buffer, remove any liquid by blotting the plate on paper towels. Washing buffer is (for 1 liter, pH 7.4): in ddw, NaCl 8.00 g, KH_2PO_4 0.20 g, Na_2HPO_4 1.15 g, KCl 0.20 g, Tween 20 0.50 g, NaN_3 0.20 g.

Conjugate

1. Conjugate: Dilute anti-CTV alkaline phosphatase conjugate 1:1000 in conjugate buffer or in TBS buffer. Conjugate buffer is, for 1 liter, pH 7.4: Tris-(hydroxymethyl) amino-methane 2.40 g, NaCl 8.00 g, PVP (Polyvinyl-pyrrolidone) MW 24,000 20.00 g, Tween 20 0.50 g, BSA (bovine serum albumin) 2.00 g, $MgCl_2$-6 H_2O 0.20 g, KCl 0.20 g, NaN_3 0.20 g.
2. Add 200 µl per well and cover plates tightly.
3. Incubate at 37 ^{o}C for 3-5 hr.
4. Wash: Empty the wells and wash 3-4 times with washing buffer, remove any liquid by blotting the plate on paper towels.

Color reaction

1. Dissolve p-nitrophenyl phosphate to 1 mg/ml in substrate buffer. Substrate buffer is for 1 liter (pH 9.8): in ddw, diethanolamine 97.00 ml, NaN_3 0.20 g (the substrate buffer available as 5x concentrate, Art. No. 110130).
2. Add 200 µl per well and incubate at ambient temperature in the dark.
3. Observe reaction and read yellow color development after 30-120 min.
4. Visually and/or read with an ELISA reader at 405 nm.