Version 19-04/24

User's Manual

Myrobalan Latent Ringspot Virus ELISA Development Kit

REF **DEIABL-PV17**

Σ **500T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

In vitro detection of Myrobalan latent ringspot virus (MLRSV) in young leaf of stone fruit tree: peach, plum, almond, and cherry trees.

Principles of Testing

The method of detection is an Enzyme-linked Immunosorbent Assay (ELISA) based on Double Antibody Sandwich (DAS) by polyclonal antibodies. Signal develops by alkaline phosphatase reaction with p-nitro phenyl phosphate.

Reagents And Materials Provided

1. Capture Antibody: Dilution Use 1:500
2. Conjugate Antibody: Dilution Use 1:500
3. Positive Control
4. Negative Control

Materials Required But Not Supplied

1. ELISA plate reader, 405 filter nm
2. Chamber 37°C
3. Manual or automatic device for rinsing ELISA plates
4. Micropipette set and disposable tips (10, 100, 200, 1000 µL)
5. ELISA plates 96 wells
6. Substrate p-nitrophenil phosphate (pNPP)
7. ELISA solutions (coating, washing, extraction, conjugate, substrate)

Storage

All kit components must be used before the expiration date stated on the vial label and stored at 4°C. The reagents are preserved by glycerol.

Reconstituted controls can be stored at -20°C and used no more than 2-3 times. In this case, a slight decrease of the signal should be expected.

Specimen Collection And Preparation

This kit is calibrated for testing plum leaf tissue. Antigen extraction is achieved by the general Elisa extraction buffer: PVP MW 24000 + Tween 20 in PBS pH 7.4.

It is recommended to prepare samples the day the assay begins. Grind plant tissue and dilute 1:15 in the

extraction buffer.

Reagent Preparation

1. The ELISA solutions are prepared according to classical ELISA formulation. The extraction buffer formulation is antigen specific.
2. The volume calculation refers to the use of 20 mL/plate/step (=200 µL/well). Consider that the washing solution volume is 60 mL/plate/washing step, while extraction buffer volume depends on sample grind technique.
3. Each antibody reagent must be diluted into its ELISA solution before using it. The calculation of the reagent volume to withdrawal from the vial is based on the dilution rate indicated on the label.

Example:

dilution 1:500 ---------- dilute 40 µL in 20 mL buffer/plate

dilution 1:1000 ---------- dilute 20 µL in 20 mL buffer/plate

dilution 1:2000 ---------- dilute 10 µL in 20 mL buffer/plate

4. **Plate lay-out and validation**

In order to validate the results, it is recommended to design the plate lay-out including:

2 replicate wells of positive control per assay

2 replicate wells of negative control per assay

2 replicate wells of blank (no antigen) per assay

1 column per plate to blank the ELISA reader against air or distilled water

Each sample should be placed into 2 replicate wells.

Assay Procedure

The shortest time to carry out the assay is 6 hours. Reading of results is made 1 hour after adding the substrate.

1. CAPTURE ANTIBODY
   - dilute specific antibody reagent (blue code) in coating buffer at the dilution read on the label
   - dispense 200 µL per well
   - incubate 2 hrs at 37°C or over night at 4°C
   - wash the plate 3 times with washing buffer at room temperature, 3 minutes soaking each time
2. SAMPLE DISTRIBUTION
   - prepare samples and controls as described in the kit instructions
   - dispense 200 µL per well according to the designed plate lay-out
   - incubate 2 hrs at 37°C or over night at 4°C
   - wash the plate 3 times with washing buffer at room temperature, 3 minutes soaking each time
3. CONIBGATE ANTIBODY

- dilute the specific conjugate antibody (green code) in conjugate buffer at the dilution read on the label
- dispense 200 µL per well
- incubate 2hrs at 37°C or over night at 4°C
- wash the plate 3 times with washing buffer at room temperature, 3 minutes soaking each time

4. SUBSTRATE
   - dilute p-nitrophenil phosphate in substrate buffer at 1 mg/mL
   - dispense 200 µL per well
   - incubate at room temperature following signal development for 1-2 hours
5. RESULTS
   - read the absorbance of the wells by the ELISA reader filter 405 nm, after blank setting
   - validate and interpret the assay outcomes against the OD readings of positive, negative and buffer controls
   - signal development can be stopped by dispensing 50 µL per well of sodium hydroxide 3 M

Note: Do not carry out more than one incubation over night at 4°C

Quality Control

The positive and the negative controls provided with the kit can be used as references (i) to verify that the assay was carried out correctly, (ii) to check the activity of reagents as prepared for the assay, and (iii) to set the test threshold.

- Positive control vial contains leaf tissue of peach GF305 / C. quinoa infected of MLRSV.
- Negative control vial contains peach leaf tissue MLRSV free.

Reconstitute freeze dried negative controls by **adding distilled water**, as stated in the label. When available, use your fresh positive control too. Process each reconstituted control as the samples.

Interpretation Of Results

1. Blank the ELISA reader and read the absorbance values.
2. The presence or the absence of virus is determined by relating the absorbance of the unknow samples to that of the threshold value. Absorbance greater or lower than the threshold value are, respectively, positive and negative results.
3. The threshold can be determined as 3 times the mean absorbance value of the negative control.
4. The mean absorbance value of positive control should be at least equal to the threshold value. If not, the results should be considered uncertain.

Precautions

In order to obtain reliable results a strict observation of the instructions and a skilful technique are required. It is recommended to:

1. Check for quality of buffers (purity, contamination, pH, etc ...)
2. Be accurate in dispensing, withdrawing and washing steps
3. Replace micropipette tips when different vials or samples are used
4. Avoid substrate contamination by using clean labware
5. Avoid using reagents remaining from other kit

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