



## **K-12 PPV-D ELISA KIT** INSTRUCTION FOR USE

### **Introduction**

The intended purpose of the diagnostic kit is the detection of plum pox potyvirus serotype Dideron (PPV-D) in apricot, peach and plum tissue. The kit can be employed to control Sharka disease and characterize PPV serotypes.

### **Principle of the assay**

The method of detection is an Enzyme-linked Immunosorbent Assay (ELISA) based on Double Antibody Sandwich Indirect (DASI). Signal is developed by alkaline phosphatase activity. Mab line 4DG5 produced by Instituto Valenciano de Investigaciones Agrarias (INVIA), Spain.

### **Specificity**

Assay specificity refers to the following bibliography:

- Cambra M., Asensio M., Gorris M.T., Perez E., Camarasa E., Garcia J.A., Moya J.J., Lopez-Abella D., Vela C., and Sanz A., 1994. Detection of plum pox potyvirus using monoclonal antibodies to structural and non-structural proteins. EPP0 Bulletin 24, 569-577.

### **Quality control**

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

- Positive control consists of leaf tissue tested for PPV-D. The presence of the virus was ascertained by ELISA, PCR and indexing. Reconstitute positive control is unable to infect woody plants.
  - Negative control consists of leaf tissue tested for PPV-D. The absence of the virus was ascertained as above.
- Controls provided with the kit are lyophilized. Reconstitute the controls in distilled water as stated in the labels. When available, test your own fresh positive control too. Process the controls as the samples.

### **Antigen extraction**

The kit is calibrated for testing leaf tissue samples of species listed above. Antigen extraction is achieved by PBS + PVP 2% + Tween 0.05%.

### **Testing time**

The shorter time required to carry out the protocol is 8 hours. Reading of results is made within 60' from substrate addition.

### **Storage of kit components**

Use reagents before expiration date (see vial label), store them at 4°C. Reagents are stabilized in glycerol.

Reconstituted controls should be stored at -20°C and reused no more than 2-3 times. Signal from reused controls might decrease drastically.

### **Components provided with the kit** (see label inside the kit package)

- Elisa reagents and controls
- Manual (instructions and protocol)
- Quality control certificate of the kit manufacture lot
- Packing list: components lots and volumes