



## **K-0305 GLRaV 1+3 ELISA KIT**

### **INSTRUCTIONS FOR USE**

#### **Introduction**

The intended purpose of this diagnostic kit is the simultaneous detection in grapevine tissue of Grapevine leafroll associated virus 1 (GLRaV-1) and Grapevine leafroll associated virus 3 (GLRaV-3). This kit has been conceived to be primarily employed in the certification programs for fanleaf complex disease control.

#### **Principle of the assay**

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.

#### **Specificity**

Assay specificity refers to the following bibliography:

- Boscia D., Greif C., Gugerli P., Martelli G.P., Walter B., and Gonsalves D., 1995. Nomenclature of grapevine leafroll associated putative closterovirus. *Vitis* 34:171-175.
- Gugerli, P. 1991. Grapevine closterovirus, p.40-51. In I.C. Rumbos, R. Bovey, D. Gonsalves, W.B. Hewitt, and G.P. Martelli (ed.), *Proceedings of the 10<sup>th</sup> Meeting of the International Council for the Study of Viruses and Virus Diseases of the Grapevine (ICVG)*. Plant Protection Institute, P.O. Box 303, 38001 Volos, Greece.

#### **Assay 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.

- 1 Positive control vial contains grapevine phloem tissue tested for GLRaV-1. The presence of the virus was ascertained by ELISA, indexing, and PCR.
- 1 Positive control vial contains grapevine phloem tissue tested for GLRaV-3. The presence of the virus was ascertained by ELISA, indexing, and PCR.
- 1 Negative control vial contains grapevine phloem tissue tested for GLRaV-1 and GLRaV-3. The absence of the viruses was ascertained as above.

Reconstitute freeze dried 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.

#### **Antigen extraction**

This kit is calibrated for testing grapevine phloem and leaf tissue. Antigen extraction is achieved by Tris-HCl buffer, pH 8.2.

#### **Testing time**

The shortest time to carry out the assay is 6 hours. Reading of results is made 1-2 hour after adding the substrate.

#### **Storage of kit components**

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.

#### **Components provided with the kit**

- Elisa reagents and controls
- Manual (instructions and protocol)
- Quality control certificate of the kit manufacture lot