



K-28A/B concave gum-associated virus (CCGaV)
ELISA KIT INSTRUCTIONS FOR USE
Protocol DAS 100 ul/well

Introduction

The intended purpose of the diagnostic kit is the detection of concave gum-associated virus (CCGaV) in citrus and apple plants. This kit has been conceived to be primarily employed to implement testing programs for citrus disease control.

Principle of the assay

The method of detection is an Enzyme-linked Immunosorbent Assay (ELISA) based on Double Antibody Sandwich (DAS), using polyclonal antibodies raised against a recombinant coat protein fragment of CCGaV (Minutolo M., et al. 2021). Signal develops by Alkaline Phosphatase reaction with p-nitrophenyl phosphate

Specificity

Assay specificity refers to the following bibliography:

1. Navarro B., Minutolo M., De Stradis A., Palmisano F., Alioto D., Di Serio F. (2018) The first phlebo-like virus infecting plants: a case study on the adaptation of negative-stranded RNA viruses to new hosts. *Molecular Plant Pathology* 19:1075-1089. DOI: 10.1111/mpp.12587
2. Minutolo M., Cinque M., Chiumenti M., Di Serio F., Alioto D., Navarro B. (2021) Identification and characterization of citrus concave gum-associated virus infecting citrus and apple trees by serological, molecular and high-throughput sequencing approaches. *Plants* 10: 2390. doi: 10.3390/plants10112390
3. Minutolo M., Cinque M., Navarro B., Alioto D., Di Serio F. (2021) Identification by serological detection methods and molecular characterization of citrus concave gum-associated virus in citrus and apple trees in Italy. 26 SIPaV Congress, September 15-17 2021. Oral communication
4. Navarro B., Minutolo M., Cinque M., Chiumenti M., Li S., Ferilli F., Alioto D., Di Serio F. Citrus concave gum-associated virus and citrus virus A: coguviruses with a wide host range coding for a weak RNA silencing suppressor. 25th International Conference on virus and one graft transmissible diseases of fruit crops (ICVF). Wageningen, The Netherlands 9-13 July 2023. Oral presentation

Assay quality control

The positive and the negative controls provided with the kit can be used as references (i) to verify that each 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 citrus leaf tissue CCGaV infected. The calibration and consistency of the control preparation was assessed against a collection of CCGaV infected citrus ascertained by PCR tests.
- Negative control vial contains citrus leaf tissue tested for CCGaV. The absence of the viruses was ascertained by PCR tests.

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

Sampling

The assay is suitable for testing:

- citrus: young and mature leaves and petiole;
- apple: young, mature leaves, petals and buds.

Antigen extraction

Antigen extraction is achieved by PBS + Tween 0,05% + PVP 2%.

Testing time

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

Storage

All kit components must be kept at 4°C and used before the expiration date read on the vial. The reagents are preserved by glycerol. When reconstituted positive controls are stored (at -20°C), a significant decrease of the signal should be expected.