Instructions for ELISA:

Double Antibody Sandwich Assay (DAS-ELISA)

Assay Principle

The Double-Antibody-Sandwich (DAS)-ELISA is one of the most common methods for serological plant pathogen detection, which consists of four basic steps:

During the first step, the surface of a microtiter plate is coated with a coating-antibody (IgG), which is directed against a specific antigen. When an antigen-containing sample is added during the second step, it will bind to the immobilized IgG, forming an IgG-antigen complex. During the third step, a complementary IgG, which is labelled with alkaline phosphatase (AP-conjugate) is added, binding to the antigen-IgG-complex and forming a double-antibody sandwich. Finally, the substrate 4-nitrophenylphosphate is applied and the alkaline phosphatase (AP) enzymatically forms yellow coloured 4-nitrophenol. The yellow colour development can be evaluated visually or measured in a spectrophotometer at 405 nm.

Handling and Storage of the Reagents

Our reagents are shipped at room temperature without ice packs. The stability at ambient temperature has been thoroughly evaluated without observing a significant loss in activity. However, after receipt the antibodies should be refrigerated (ca. 4°C) for long-term storage. Once opened, we recommend using the reagents within 5 months.

Preparation of Samples

Leaves and juicy samples can be squeezed in simple plastic bags using commercial homogenizers, a hammer, juice presses etc.. Some fibrous tissues (mycelia of fungi, woody plant parts) need to be grinded with sand in mortars to assure proper crushing of the cell walls.

It is very important to dilute the samples sufficiently, i.e. min. 1:20 to 1:30. Positive samples - when used too concentrated - sometimes give no or too low signals. At the same time negative samples can exhibit high background reactions.

Please consult official diagnostic protocols published by plant health organisations such as EPPO (European Plant Protection Organisation) or ISTA (International Seed Testing Association) for sampling and sample preparation recommendations.

Evaluation

We strongly advise to add the positive and negative controls to each plate for verification of a strong positive and a low negative reaction.

To determine potential background of healthy plants, add fresh non-infected extracts of the tested species and tissue at the same dilution to each plate. However, the positive/negative threshold needs to be determined by the user, as it depends on many factors, such as plant species and its physiological conditions (e.g. tissue type, age).

Performing the Assay

Coating plates with IgG 1

- Dilute IgG from original vial **1:200*** in Coating Buffer, mix gently but thoroughly.
- Add **0.2 ml*** of the IgG working solution to one well of the ELISA plate and cover plate tightly.
- Incubate at 37°C for 4 hours or refrigerate overnight
- Remove IgG by washing** with Wash Buffer.

Sample preparation and application 2

- Homogenize samples with ratio of **1:20 w/v** or as recommended in Conjugate/Sample Buffer.
- Add **0.2 ml***of sample to one well of the ELISA plate and cover plate tightly.
- Incubate overnight in the refrigerator.
- Remove sample by washing** with Wash Buffer.

Application of IgG-AP-conjugate 3

- Dilute Conjugate **1:200*** from original vial in **Conjugate/Sample Buffer**, mix gently but thoroughly.
- Add **0.2 ml*** of the conjugate working solution to one well of the ELISA plate and cover plate tightly.
- Incubate at 37°C for 4 hours or refrigerate overnight
- Remove conjugate by washing** with Wash Buffer.

Enzymatic Reaction 4

- Prepare substrate solution directly before use.
- Add **0.2 ml** of the substrate solution to one well of the ELISA plate and incubate at ca. 25°C in the dark.
- After 1 and 2 hours of substrate incubation, evaluate the reaction visually or read photometrically (405 nm).

** **ATTENTION**: unless stated otherwise in the product specification sheet*
*** **Washing:** After each incubation step, the reagents are removed with Wash Buffer by four washing cycles using an automated washer or manually.*

Buffer Formulations

We recommend to make buffer solutions as fresh as possible or to prepare aliquots and store them frozen until needed. Check pH and adjust with NaOH or HCl. 0.01% sodium azide can be added, if desired.

Coating Buffer	1.59 g Na_2CO_3 2.93 g $NaHCO_3$	**Fill to 1 Liter** Adjust pH to 9.6
Wash Buffer	8.0 g NaCl 2.9 g Na_2HPO_4 x 12 H_2O 0.2 g KH_2PO_4 0.2 g KCl 0.5 ml Tween 20	**Fill to 1 Liter** Adjust pH to 7.2 - 7.4
Conjugate/ Sample Buffer	Add to Wash Buffer formulation: 20 g polyvinyl pyrrolidone (K10-K40) 2 g bovine serum albumin	**Fill to 1 Liter** Adjust pH to 7.4
Substrate Buffer	97 ml diethanolamine 0.2 g $MgCl_2$ x 6 H_2O	**Fill to 1 Liter** Adjust pH to 9.8
Substrate Solution	1 mg/ml 4-nitrophenyl-phosphate-di-Na-salt in Substrate Buffer	**Prepare this solution just prior to use!**