Version 29-08/24

User's Manual

Wheat Dwarf Virus (WDV) ELISA Kit

REF **DEIAPV296**

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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.

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PRODUCT INFORMATION

Intended Use

For detection of Wheat Dwarf Virus (WDV) in test samples.

Reagents And Materials Provided

The Double Antibody Sandwich (DAS) ELISA is one of the most common methods forserological 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. During the fourth step the substrate 4-nitrophenyl-phosphate is applied and the alkaline phosphatase (AP) enzymatically forms yellow coloured 4-nitrophenol. The colour development can be evaluated visually or measured in a spectrophotometer at 405 nm.

Materials Required But Not Supplied

1. Antibody (lgG): 0.5 ml
2. Antibody-AP-conjugate: 0.5 ml
3. Positive Control: 1 vial
4. Negative Control: 1 vial
5. Coating Buffer: 1 liter
6. Wash Buffer: 1×5 liter
7. Conjugate/Sample Buffer: 2×1 liter
8. Substrate Buffer (5×): 1×25 ml
9. Blocking Milk Powder: 10 g
10. Substrate Tablets: 5×20 mg
11. Tween* 20: 5 ml
12. High-binding ELISA plates: 5 plates
13. Sealing Cover: 5 sheets

Storage

The stability at ambient temperature has been evaluated without observing a significant loss in activity. However, after receipt the antibodies must be kept refrigerated (ca.4°C). Once opened, we recommend using the reagents within 5 months. Our DAS-ELISA reagents are standardized for use at a dilution of 1:200 and a test volume of 200 µL/well.

Negative Controls must be kept refrigerated. Once dissolved, it is advised to aliquot the controls and store them frozen until use. Store them frozen until use. However, depending on the stability of the particular

pathogen, prolonged storage might result In reduced activity. Repeated freezing and thawing should be avoided as it can result in loss of activity.

Analytical data and other product specifications can be derived from the Product Specification sheet that is included with each reagent set.

Specimen Collection And Preparation

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

Reagent Preparation

1. **Coating Buffer:** Dissolve the content of the sachet in approx 900 ml distilled water, adjust pH to 9.6 and fill up to 1L. Store refrigerated until use. Keep frozen in glass bottles for long term storage.
2. **Wash Buffer:** Dissolve content of the the sachet in 5 L of distilled water. Add 2.5 mL of Tween 20 (0.05% final concentration) adjust pH to 7.4. Store refrigerated Keep frozen for long-term storage.
3. **Conjugate/sample Buffer**: Dissolve content of the sachet in approx 900 ml water and adjust pH to pH 7.4 with sodium hydroxide. Add 05 ml Tween 20 and fill up to 1L. If desired 0.01% sodium azide can be added. Store refrigerated for not longer than 1 week. We recommend freezing aliquots and use the buffer as fresh as possible.
4. **Conjugate Buffer with 2% Blocking milk for conjugate dilution:** Dissolve the content of the Blocking Milk bag in 0.5 L of the prepared Conjugate/Sample Buffer solution (for sample extraction, see above) Sore refrigerated for no longer than 1 week. We recommend freezing aliquot and use the buffer as fresh as possible.
5. **Substrate Buffer:** Dilute 25 mL of 5× concentrate with approx. 100 mL of water. Adjust pH to 9.8 before filing up to 125 mL. Store refrigerated. Keep frozen in glass bottles for long-term storage.
6. **Substrate Solution:** Dissolve substrate, equivalent to 1 mg/ml in diluted substrate buffer (1×) directly before use.

Our buffer formulations do not contain sodium aide.

7. Preparation of Antibody Working Solution:

Our antibodies are pre-diluted to minimize deviations from pipetting errors. Please follow our pipetting scheme to obtain the working dilution.

No. of wells to be filled	IgG or Conjugate Stock Solution from original vial	Coating Buffer (for IgG Dilution) Conjugate/Sample Buffer with 2% Blocking Milk (for Conjugate Dilution)
480 (5 plate)	500 µL	20 mL

Assay Procedure

1. **Coating Plates with Antigen-specific Antibodies (IgG)**

· Dilute lgG from original vial 1:200* in Coating Buffer mix gently but thoroughly.

· Add 0.2 ml*of the lgG working solution to one well of the ELISA plate.

· Tightly cover plate with sealing tape.

· Incubate at 37°C for 4 hours or overnight in the refrigerator.

· Remove IgG with Wash Buffer by four washing cycles using an automated washer or by manual washing.

2. **Sample Preparation and Application**

· Homogenize samples with ratio of 1:20 w/v in Conjugate/Sample Buffer.

· Dissolve Positive or Negative Controls in ca.2 ml Conjugate/Sample Buffer.

· Add 0.2 ml*of sample extract or Control solution to one well of the ELISA plate.

· Tightly cover plate with sealing tape.

· Incubate over night in the refrigerator.

· Remove sample with Wash Buffer by four washing cycles using an automated washer or by manual washing.

3. **Application of Antibody-AP-conjugate**

· Dilute Conjugate 1:200* from original vial in Conjugate/Sample Buffer,containing 2% blocking Milk, mix gently but thoroughly.

· Add 0.2 ml of the Conjugate working solution to one well of the ELISA plate.

· Tightly cover plate with sealing tape.

· Incubate at 37°C for 4 hours or overnight in the refrigerator.

· Remove conjugate with Wash Buffer by four washing cycles using an automated washer or by manual washing.

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)

Specificity

NA

Precautions

Washing

Careful washing is also a crucial point. Best and Reproducible results are obtained by using automated ELISA washers. Washing by hand represents the most gentle method but often leads to irreproducible results. Automated washers on the other hand have to be optimized because the pressure of washing buffer injection into the wells is often too high. Wrong pressure and too extensive washing can lead to Weak results. Also, daily cleaning of the washer is Important for reproducible function.

Sample Volume

Our ELISA reagents are standardized for a sample volume of 0.2 ml/well, if not indicated otherwise in the product manual. We do not recommend smaller volumes and cannot guarantee the OD value will meet our product specifications if other volumes are used.

Storage of plates between Individual ELISA steps

This is important in case many plates have to be handled simultaneously. We recommend to store plates covered with a tape in the refrigerator until use.

ELISA Buffers

Do not use other buffer formulations besides the ones given in our protocol because our ELISA reagents are standardized using these buffers.

It Is important to adjust and control the pH value of the buffers. The pH of the substrate buffer is especially important, as the enzymatic activity of the alkaline phosphatase has a very small optimum at pH 9.8. Deviations lead to a considerable reduction of the OD values. Especially sample and conjugate buffers are prone to microbial growth, if stored in the refrigerator which can lead to odd ELISA results. Use ready-to-use buffer solutions as fresh as possible. Store liquid in the freezer until use. Alkaline buffers should be stored in glass bottles.

Incubation Temperatures and Times

We refer you to our standard protocol. In case there are practical reasons against this scheme, it is in the responsibility of the user to test whether modified incubation temperatures and times lead to the same reliable results as the standard protocol.