Creative Diagnostics®

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User's Manual

Wheat Yellow Mosaic Virus (WYMV) ELISA Kit

REF **DEIAPV300**

Σ **500T/1000T/5000T**

RUO

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.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The test can be used to detect WYMV in host plants.

General Description

Wheat yellow mosaic virus is a plant pathogenic virus of the family Potyviridae.

Principles of Testing

The enzyme-linked immunosorbent assay (ELISA) is a serological solid-phase method for identification of diseases based on antibodies and color change in the assay. In this method, specific antibodies are used to coat the microtitre plate, which then trap the target epitopes (antigens) from the viruses, bacteria and fungi. An enzyme-labelled specific antibody conjugate is then used for the detection. The detection can be visualized or measured in a computer controlled plate reader based on color changes resulting from the interaction between the substrate and the immobilized enzyme.

Reagents And Materials Provided

Coating Antibody: Specific capture polyclonal antibody (PAb) or monoclonal antibody (MAb) for coating ELISA plate.

Detecting Conjugate: Specific antibody enzyme conjugate, or anti-species antibody enzyme conjugate.

ELISA Plate: Empty ELISA plate with high binding capacity. The plate should be used for our ELISA Reagents to achieve a maximum test results.

Controls: Positive control and negative control

Buffers: Coating buffer, sample buffer, enzyme conjugate buffer, washing buffer, substrate buffer and PNP tablets.

Instruction: Specific test instructions for each of the three different ELISA Formats.

Storage

All reagent components should be stored at the recommended temperature to assure their full shelf life. The kit should be used within six months of purchase.

Reagent Preparation

Buffer Formulations:

Coating Buffer

Sodium carbonate (anhydrous) 1.60 g

Sodium bicarbonate 2.92 g

Sodium azide 0.2 g

Dissolve in distilled water and make to 1000 ml. Adjust pH to 9.6. Store at 4°C.

PBST Buffer

Sodium phosphate, dibasic, (anhydrous) 1.15 g

Potassium phosphate, monobasic (anhydrous) 0.2 g

Sodium chloride 8.0 g

Potassium chloride 0.2 g

Tween-20 0.5 g

Dissolve in distilled water and make to 1000 ml. Adjust pH to 7.3.

SB1 Buffer

Powdered egg (chicken) albumin, Grade II 2.0 g

Polyvinylpyrrolidone (PVP) MW, 24-40,000 10.0 g

Sodium sulfite (anhydrous) 1.3 g

Sodium azide 0.2 g

Tween-20 10.0 g

Dissolve in 1000 ml of 1X PBST. Adjust pH to 7.3. Store at 4°C.

ECB1 Buffer

Bovine serum albumin (BSA) 2.0 g

Polyvinylpyrrolidone (PVP) MW 24-40,000 10.0 g

Sodium azide 0.2 g

Dissolve in 1000 ml of 1X PBST. Adjust pH to 7.3. Store at 4°C.

PNP Buffer

Diethanolamine 97.0 ml

Magnesium chloride 0.1 g

Sodium azide 0.2 g

Dissolve in 800 ml distilled water. Adjust pH to 9.8 with hydrochloric acid. Adjust final volume to 1000 ml with distilled water. Store at 4°C.

Assay Procedure

Preparing For The Test

1. Check all the components in the package of ELISA Reagents.
2. Prepare all buffer solutions according to the attached buffer formulations.
3. Make sure all laboratory equipments and facilities required for the test are ready.

4. Prepare a humid box for incubation steps.
5. Make a copy of the attached recording sheet and create a loading diagram by recording the locations of your samples, controls, and other reagents needed.

Coating Plate With Antibody

1. Lay out all items that will be required for the plate coating step before beginning. Prepare coating antibody in a container made of glass, polyethylene or any material that does not readily bind coating antibody.
2. Coat the plate immediately after preparing the coating antibody. Some coating antibody can be lost if too much time elapses between diluting the coating antibody and coating the plate.
3. The volume of coating buffer required depends on the number of test wells used; 100 µl is needed per test well. One way to estimate the volume needed is to prepare 1 ml of coating buffer for each 8-well strip used, or 10 ml for each 96-well plate.
4. Dilute the concentrated coating antibody into coating buffer at the dilution given on the label. Mix well.
5. Always prepare coating antibody immediately before use.
6. Pipette 100 µl of coating antibody into each well.
7. Incubate the plate in a humid box for overnight in the refrigerator (4°C) or 4 hours at room temperature (21-24°C).

Preparing Samples

1. Select symptomatic and/or infective tissues for the test. Leaf tissue is often used in ELISA testing. Plant tissues such as stem, sprout, seed, tuber, root and others can also be used.
2. We suggest that each test well be used for only one sample. In some cases, composites of up to ten leaves per test well can be used to make testing more economical. However, too many plant samples per well can reduce the sensitivity of the test.
3. CD's SB1 buffer can be used as extraction buffer for most of the plant samples. However, other buffers are also recommended for some plant species.
4. Grind sample with a mortar and pestle, or other grinding device. If you are using a mortar and pestle, wash and rinse it thoroughly between samples.
5. If you extract plant sap, dilute the sap into sample extraction buffer at a ratio of 1:10 (sap volume: buffer volume). Or you can grind plant tissue in extraction buffer at a 1:10 ratio (tissue weight: Extraction Buffer volume).
6. If you have any questions about sampling, sample preparation, or the appropriate extraction buffer for your samples, please contact CD, Inc.

Plate Washing

1. Wash the plate when the incubation is complete. Use a quick flipping motion to empty the wells into a sink or waste container.
2. Wash the plate by filling the wells with PBST, then quickly emptying them again. Repeat 4 to 6 times.
3. To remove drops of PBST from the wells after washing, hold the frame upside down and tap firmly on a folded paper towel.

Sample Dispensing and Incubation

1. About 100 µl of diluted sample extract is needed per test well. Always have an additional amount to assure easy dispensing. A convenient way to prepare this diluted sample is to measure 100 µl of undiluted sap into a small test tube, then add 1 ml of extraction buffer.

2. Following your loading diagram on your recording sheet, dispense 100 µl of prepared sample into sample wells. Dispense 100 µl of positive control into positive control wells, and dispense 100 µl of negative control or extraction buffer into negative control wells.
3. Put the plate inside the humid box and incubate for 2.5 hours at room temperature (21-24°C) or overnight in the refrigerator (4°C).

Preparing Enzyme Conjugate

1. Always make enzyme conjugate solution within 10 minutes before use. Prepare the enzyme conjugate, using CD's ECB1 buffer and a cleaning container.
2. The volume of ECB1 buffer required depends on the number of test wells used; 100 µl are needed per test well. To estimate the volume needed, prepare 1 ml for each 8-well strip used, or 10 ml for each 96-well plate.
3. The volume of enzyme conjugate required for each test is calculated based on the volume of ECB1 buffer used and on the dilutions given on the bottles. Use a new, sterile pipette tip and change the tip for each pipetting to prevent contamination.
4. First dispense appropriate volume of ECB1 buffer into a cleaning container, then add enzyme conjugate according to the dilution given on the label. Mix the conjugate solution thoroughly. It is important to mix the enzyme conjugate well for a consistent test result.
5. Prepare enzyme conjugate just before use. Keep the prepared enzyme conjugate at a safe place and use it after washing the plate.

Washing Plate

1. Wash the plate when the incubation is complete. Use a quick flipping motion to empty the wells into a sink or waste container without mixing the contents.
2. Wash the plate by filling the wells with PBST, then quickly emptying them again. Repeat 6 to 8 times.
3. To remove drops of PBST from the wells after washing, hold the frame upside down and tap firmly on a folded paper towel.

Enzyme Conjugate Incubation

1. Dispense 100 µl of prepared enzyme conjugate per well for all test wells.
2. Incubate the plate in the humid box for 2.5 hours at room temperature (21-24°C).

Preparing Substrate Solution

1. Concentration of PNP in substrate is 1 mg/ml. Each PNP tablet will make 5 ml of PNP solution, which is enough for 48 test wells or five 8-well strips.
2. Do not touch the PNP tablets or expose the PNP solution to strong light. Light or contamination could cause background color in negative wells.
3. Prepare PNP substrate about 10-15 minutes before the end of the above incubation step. Measure 5 ml of PNP buffer for each tablet, then add the PNP tablets to the buffer. Mix by vortexing or stirring to let the PNP tablet fully dissolve in the buffer.

Washing plate

Wash the plate 6 to 8 times with PBST as instructed above.

Incubation With Substrate

1. Dispense 100 µl of PNP substrate solution per well.

2. Incubate the plate for 30 to 60 minutes in a humid box at room temperature (21-24°C).
3. To stop reaction, add 50 µl of 3M sodium hydroxide to each well. This step is optional. The plate can be interpreted visually or with a plate reader without adding the stop solution.

Quality Control

Negative controls are made from a healthy host plant and are tested for the absence of the respective pathogen. They are lyophilized and sufficient for 10 test wells. Positive controls are made from infected plant material or bacterial cultures, if not stated otherwise. They are lyophilized and sufficient for 10 test wells. Inactivated positive controls are additionally tested for the absence of infectivity. Unspecific non-pathogenic 'method' controls are sold for some quarantine pathogens.

Interpretation Of Results

Test results can be examined by eye, or measured on a plate reader at 405 nm.

Development of yellow color in test wells indicate positive results. Wells in which there is no significant color development indicate negative results. Test results are valid only if positive control wells give a positive result and negative control wells remain clear.

Results may be interpreted after more than 60 minutes of incubation as long as negative control wells remain virtually clear.

Sensitivity

Sensitivity of the ELISA is relative high. The virus can be consistently detected in infected plant tissues diluted at 1:810 - 1:2430.

Specificity

Reaction of the ELISA is moderately strong. Optical Density at 405nm is in a range of 0.800 - 1.800 depending on the virus titer in the samples tested.

This test system has no cross reaction with healthy plant tissues tested. Background is very low on the negative control wells.

Precautions

1. Always wash hands thoroughly after using this product. Prevent direct skin and eye contact with, or ingestion of, product components. Obtain medical attention in case of accidental ingestion of reagent components.
2. All reagent components should be stored at the recommended temperature to assure their full shelf life. Do not store prepared working solution from day to day.

References

Hibino, H., Usugi, T. and Saito, Y. (1981). Ann. Phytopath. Soc. Japan 47: 510.