Version 10-11/24

**User's Manual**

# Tomato Mosaic Virus (ToMV) ELISA Kit

REF **DEIAPV10**

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.

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# PRODUCT INFORMATION

## Intended Use

The test can be used to detect ToMV in infected solanaceous crops.

## General Description

ToMV is found worldwide and economically damaging in glasshouse and outdoor tomatoes and many other plants. ToMV can cause yellowing and stunting of tomato plants, resulting in loss of stand and reduced yield. In addition, the virus may cause uneven ripening of fruit, further reducing yield. The virus is readily spread by handling and cultural operations. It also contaminates seeds and soil, but no natural vector is known. The best measure to control and reduce infection is to use certified disease-free seed and remove any infected plants, including the roots. Remove Also, discard any plants near those affected.

## 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

**1. Coating Antibody:** 0.25 mL. Specific capture polyclonal antibody (PAb) or monoclonal antibody (MAb) for coating ELISA plate.

**2. Detecting Conjugate, alkaline phosphatase:** 0.25 mL.

**3. ELISA Plate:** 5×96T. Empty ELISA plate with high binding capacity. The plate should be used for our ELISA Reagents to achieve a maximum test results.

**4. Coating Buffer 10×:** 6 mL

**5. Sample Buffer:** 11.6g

**6. Conjugate Buffer 10×:** 6 mL

**7. PBST Buffer (Powder):** 28.7g

**8. Tween-20:** 7.0g

**9. PNP Buffer (5×):** 10 mL

**10. PNP Tablet:** 10 Tablets

## Storage

Store all buffers at 2-8°C, except for PNP tablets at -20°C under dark.

## Reagent Preparation

### 1. Preparing 1× Buffer from Concentrate:

To prepare the 1× buffer, dilute the concentrated buffers at 1:10 (Coatingand Conjugate buffers), and 1:5 (PNP buffer) with D.$H_2O$ . Pour all the content into D.$H_2O$ and stir the diluted buffers for 10-30 minutes for mixing well anddissolving completely. If only part of the concentrated buffer being used, stir or mix the buffer first before pipetting.

### Preparing 1× Buffer from Powder:

To prepare the 1× buffer, dissolve the powder buffer into D.H2Oat the ratioonthe table below. For sample buffer, mix the powder with small amount of D.H2Ointo a paste (no lumps) before adding more D.$H_2O$. Stir for 10-30 minutesfor dissolving completely, and make up to final volume.

| | 1×Sample buffer | | | 1×TBST Buffer | | |
|---|---|---|---|---|---|---|
| Buffer Powder | 11.6g | 23.2g | 115.7g | 28.7g | 57.3g | 286.5g |
| Tween-20 | 5.0g | 10.0g | 50.0g | 1.5g | 3.0g | 15.0g |
| Final Volume | 500 mL | 1000 mL | 5000 mL | 3000 mL | 6000 mL | 30000 mL |

### Storage of 1× Buffer

Store the prepared 1× buffers at 2-6°C refrigerator. The 1x buffers can be store up to 3 months.

### Using the buffers

For detail information on using the buffers for preparing reagents, refer to the Assay Procedure.

### Control Preparation

To prepare the control, use the sample extraction buffer for the test system and the protocol for which the control will be used. The volume on the control label is the amount of sample extraction buffer required to prepare the control. Add this amount of sample extraction buffer to the vial to prepare the control. Mix the rehydrated control in the vial by gently shaking until fully dissolved.

### Making Aliquots

The prepared control can be used immediately, or divided into aliquots and stored frozen (-10 to - 40°C). Each aliquot should be sufficient for at least one use. For example, if you will use this control in one well each time you run the test, prepare 120 ul aliquots. Prepare 220 µl aliquots if you will use the control in two wells.

Control aliquots must be kept frozen until just before use. Do not refreeze controls once they have been thawed.

## Assay Procedure

### Preparing For The Test

Check all the components in the package of ELISA Reagents.

Prepare all buffer solutions according to the attached buffer formulations.

Make sure all laboratory equipment and facilities required for the test are ready.

Prepare a humid box for incubation steps.

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**

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. 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.

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.

Dilute the concentrated coating antibody into coating buffer at the dilution given on the label. Mix well.

Always prepare coating antibody immediately before use.

Pipette 100 µl of coating antibody into each well.

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**

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.

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.

The SB1 buffer can be used as extraction buffer for most of the plant samples. However, other buffers are also recommended for some plant species.

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.

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).

**Plate Washing**

Wash the plate when the incubation is complete. Use a quick flipping motion to empty the wells into a sink or waste container.

Wash the plate by filling the wells with PBST, then quickly emptying them again. Repeat 4 to 6 times.

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**

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.

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.

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**

Always make enzyme conjugate solution within 10 minutes before use. Prepare the enzyme conjugate, using ECB1 buffer and a cleaning container.

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.

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.

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.

Prepare enzyme conjugate just before use. Keep the prepared enzyme conjugate at a safe place and use it after washing the plate.

**Washing Plate**

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.

Wash the plate by filling the wells with PBST, then quickly emptying them again. Repeat 6 to 8 times.

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**

Dispense 100 pu of prepared enzyme conjugate per well for all test wells.

Incubate the plate in the humid box for 2.5 hours at room temperature (21-24°C).

**Preparing Substrate Solution**

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.

Do not touch the PNP tablets or expose the PNP solution to strong light. Light or contamination could cause background color in negative wells.

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**

Dispense 100 µl of PNP substrate solution per well.

Incubate the plate for 30 to 60 minutes in a humid box at room temperature (21-24 °C).

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.

## Calculation

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.

## Specificity

There is no cross reaction with healthy plant tissues. Background is low on all of the negative control wells.