Plum pox virus (PPV) Reagent Set
ELISA for the detection of PPV-C, D, EA, M, and W
Alkaline phosphatase label,
Catalog number: SRA 31505

List of contents

<table>
<thead>
<tr>
<th>Lot number</th>
<th>Item</th>
<th>96 wells</th>
<th>500 wells</th>
<th>1000 wells</th>
<th>5000 wells</th>
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<tbody>
<tr>
<td></td>
<td>Capture antibody</td>
<td>0.150 ml</td>
<td>0.275 ml</td>
<td>0.525 ml</td>
<td>2.525 ml</td>
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<tr>
<td></td>
<td>Alkaline phosphatase enzyme conjugate</td>
<td>0.150 ml</td>
<td>0.275 ml</td>
<td>0.525 ml</td>
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<td></td>
<td>Positive Control</td>
<td>10 strips</td>
<td>10 strips</td>
<td>10 strips</td>
<td>50 strips</td>
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<tr>
<td></td>
<td>RUB3 Enzyme conjugate diluent</td>
<td>11 ml</td>
<td>55 ml</td>
<td>110 ml</td>
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*The above items should be stored at 4°C.*

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<tr>
<th></th>
<th>Tween 20</th>
<th>30 ml</th>
<th>2 X 30 ml</th>
<th>4 X 30 ml</th>
<th>1 X 250 ml</th>
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<tr>
<td></td>
<td>General extract buffer 4 (GEB4) powder (see Limitations section below)</td>
<td>82.5 g</td>
<td>82.5 g</td>
<td>165 g</td>
<td>3 X 165 g</td>
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<tr>
<td></td>
<td>96-well microtiter plates, strip or solid</td>
<td>1 strip</td>
<td>5 solid</td>
<td>10 solid</td>
<td>50 solid</td>
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</tbody>
</table>

*The above items can be stored at room temperature.*

Materials required, but not provided

- Carbonate Coating Buffer (formulation on page 6)
- PBST Wash Buffer (formulation on page 6)
- PNP Substrate Buffer (formulation on page 6)
- PNP Substrate Tablets (ACC 00404)
- Buffer packs containing the above required items can be purchased from Agdia (ACC 00113)
- Distilled or purified water
- Paper towels
- Micropipette
- Micropipette tips
- Sample grinding device such as:  
  - Agdia sample mesh bag (ACC 00930)
  - Agdia tissue homogenizer (ACC 00900)
  - Mortar and pestle
- Airtight container for incubations
- Postcoat 10 buffer (ACC 00650) optional

Limitations

Expiration: This test should be used within 1 year of purchase.

Storage: Test results may be weak or the test may fail if storage instructions are not followed properly.

Buffers: Do not store 1X buffers from day to day. Buffers should be warmed to room temperature prior to use.

GEB4: Agdia has supplied sufficient powdered buffer to prepare 15 liters of General extract buffer 4 (GEB4). This volume will easily accommodate the general testing scheme of using duplicate wells for each sample, where each sample weighs 0.5 g or less. Should your process differ from this scheme calculate the volume of buffer needed. Additional GEB4 buffer powder can be purchased from Agdia, catalog number ACC 00380/082.5 or ACC 00380/0165.
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Dilutions: Read all labels carefully prior to preparing solutions to assure proper antibody concentrations. All antibody dilutions have been optimized for the greatest possible sensitivity and specificity based on available isolates and hosts. Using dilutions other than those listed can lead to potential false positives or false negatives.

Sample Dilution: ELISA performance is very dependent on the proper sample (tissue weight in g: buffer volume in ml) dilution.

Sampling: PPV virus in infected trees can be unevenly distributed in the tree and will be at lower concentrations in the summer as temperatures increase. These factors can limit the ability of detecting the virus. Sampling from throughout the tree canopy, selecting symptomatic tissue and testing in spring and early summer will increase the chances of detecting the virus.

Precautions

Prevent direct skin and eye contact with, or ingestion of, product components. Obtain medical attention in case of accidental ingestion of kit components. Always wash hands thoroughly after using this product.

Technical Service

If you have any questions about using this kit, please contact Agdia, Inc. Monday – Friday by phone (574-264-2014 or 800-622-4342) or by email (info@agdia.com).

Intended Use

This test can be used to detect the Plum pox virus in leaves, fruit, and flowers of stone fruits. The PPV DAS ELISA test detects the following PPV isolates: PPV-C, PPV-M, PPV-D, PPV-EA and PPV-W.

Test Principle

The PPV test system uses a direct, double antibody sandwich protocol known as DAS ELISA. Antibodies specific to PPV are coated to the testwells of a microplate. Next, samples are added to the microplate. If PPV is present in the sample, it is bound by the antibodies and captured on the microplate during the incubation period. After incubation, the plate is washed to remove unbound sample. An enzyme conjugate solution, containing a polyclonal antibody conjugated to alkaline phosphatase, is added and binds to any captured PPV. After incubation the plate is washed to remove any unbound conjugate. This final binding creates a sandwich of the target analyte between the two specific antibodies. PNP substrate (pNPP) is added to the microplate. If the alkaline phosphatase conjugate is present a yellow color will be produced indicating the presence of PPV. Buffer wells and negatives should remain colorless. The color reactions can be observed visually or measured with a spectrophotometer.

References


Coat testwells of ELISA plate

1. Prepare humid box

Prepare a humid box by lining an airtight container with a wet paper towel. Keeping testwells in a humid box during incubation will help prevent evaporation.

2. Prepare capture antibody

Note: 96 testwell reagents have a different dilution ratio than larger reagent sets. Please read label on capture antibody bottle for appropriate dilution ratio.

Note: All antibodies and enzyme conjugates should be prepared in a container made of a material such as polyethylene or glass that does not readily bind antibodies. Do not use polystyrene, polypropylene or polycarbonate.

The capture antibody is provided as a concentrated solution and must be diluted with carbonate coating buffer before use. The recommended antibody to buffer ratio is given on the label.

Prepare the volume of carbonate coating buffer needed for the test. You will need 100 µl of carbonate coating buffer for each test well you are using. A full plate will require about 10 ml. Then, add the appropriate volume of concentrated capture antibody to the carbonate coating buffer at the dilution on the label.

Example 1: If the dilution given on the bottle of concentrated capture antibody is 1:200, and you are preparing 10 ml of capture antibody solution, you should mix 10 ml of carbonate coating buffer with 50 µl of the concentrated capture antibody. Mix the prepared capture antibody solution thoroughly and use immediately.

Example 2: If the dilution given on the bottle of concentrated capture antibody is 1:100, and you are preparing 10 ml of capture antibody solution, you should mix 10 ml of carbonate coating buffer with 100 µl of the concentrated capture antibody. Mix the prepared capture antibody solution thoroughly and use immediately.

3. Coat plate

Pipette 100 µl of the prepared capture antibody into each well.

4. Incubate plate

Incubate the plate in a humid box for 4 hours at room temperature or overnight in the refrigerator (4°C). Do not store coated plates longer than 24 hours. If long term storage is desired, contact Agdia about postcoat buffers.

5. Wash plate

Empty the wells into a sink or waste container. Fill the wells completely with 1X PBST, and then quickly empty them again. Repeat 2 times.

Hold the plate upside down and tap firmly on a folded paper towel to remove excess liquid.

Note: Use freshly coated plates immediately. If you would like to store the plates for future use it is necessary to apply Postcoat 10 (ACC 00650) available separately from Agdia.
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General extract buffer 4 (GEB4)

GEB4 is used to dilute and extract samples.

- Buffer powder: 33 g
- Distilled water: 1000 ml (1 liter)
- Tween 20: 20 ml or 20 g

To make 1 liter of GEB4 sample extract buffer, add about 50 ml of water to the powder and mix into a smooth slurry. While mixing, slowly add the remaining volume of water. Add Tween 20 to the solution. Stir for 30 minutes.

Agdia recommends preparing only as much buffer as is needed for one day. Those who store buffers outside of this recommendation are advised to add sodium azide (Sigma S-2002) to 1X liquid buffers at a rate of 0.2 g per liter (0.02%).

**Grind and dilute samples**

Samples must be ground and diluted with General extract buffer 4 (GEB4).

You can use Agdia’s sample mesh bags (ACC 00930), Agdia’s tissue homogenizer (ACC 00900), a mortar and pestle, or other grinding devices to grind samples. If you are using a mortar and pestle, wash and rinse it thoroughly between samples.

Use no more than eight Prunus leaves per sample. Stack the leaves with all of the petioles at one end. Remove the petioles and discard them. Use 0.5 g of the basal portion of the stacked leaves.

Grind plant tissue in GEB4 extraction buffer at sample to buffer ratio of a 1:10 (tissue weight in g: buffer volume in ml).

*Example:* A sample weighing 0.5 grams requires 5 ml of GEB4 buffer.

You will need 100 µl of the ground and diluted sample per testwell, plus an additional amount to assure easy dispensing. It is recommended to use two testwells per sample.

**Positive control**

Before opening the container of control strips, let the container warm at room temperature for 15 minutes. This maintains the shelf life of the strips. Do not allow the container to remain open. Keep tightly sealed between uses.

Each control strip requires 500 µl of GEB4 buffer. Dispense the required amount of GEB4 sample extract buffer into tubes or other container. Dip the pad end of the strip into the buffer and let it sit for 5 minutes. Use the strips to stir the buffer before using.

The control strip can only be used once, after which it should be discarded. Do not store the positive control solution. It should be discarded after one day.

**Test Procedure**

1. **Dispense samples**

Following your loading diagram, dispense 100 µl of prepared sample into sample wells. Dispense 100 µl of positive control into the positive control wells, 100 µl of negative tissue into the negative control wells, and 100 µl of extraction buffer into the buffer wells.
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2. Incubate plate
Set the plate inside the humid box and incubate for 2 hours at room temperature or overnight in the refrigerator (4°C).

3. Prepare enzyme conjugate

Note: 96 testwell reagents have a different dilution ratio than larger reagent sets. Please read label on enzyme conjugate bottle for appropriate dilution ratio.

The alkaline phosphatase enzyme conjugate is supplied as a concentrate and must be diluted with RUB3 enzyme conjugate diluent before use.

Dispense the appropriate volume of RUB3 enzyme conjugate diluent into a dedicated container. You will need 100 µl of diluent for each testwell you are using. Then, add the alkaline phosphatase enzyme conjugate according to the dilution given on the label.

Example 1: If the dilution given on bottle of concentrated alkaline phosphatase enzyme conjugate is 1:200, and you are preparing 10 ml of enzyme conjugate solution, you should first dispense 10 ml of RUB3 buffer. Then, add 50 µl of the concentrated enzyme conjugate to the RUB3 buffer.

Example 2: If the dilution given on bottle of concentrated alkaline phosphatase enzyme conjugate is 1:100, and you are preparing 10 ml of enzyme conjugate solution, you should first dispense 10 ml of RUB3 buffer. Then, add 100 µl of the concentrated enzyme conjugate to the RUB3 buffer.

After adding the enzyme conjugate, mix thoroughly. It is important to mix the enzyme conjugate solution well.

4. Wash plate
When the sample incubation is complete, wash the plate. Use a quick flipping motion to dump the wells into a sink or waste container without mixing the contents.

Fill all the wells completely with 1X PBST, and then quickly empty them again. Repeat 7 times.

After washing, hold the frame upside down and tap firmly on a folded paper towel to remove all droplets of wash buffer.

Inspect the testwells. All wells should be free of plant tissue. If tissue is present repeat the wash step and tap firmly on a paper towel.

5. Add enzyme conjugate
Dispense 100 µl of prepared enzyme conjugate per well.

6. Incubate plate
Incubate the plate in the humid box for 2 hours at room temperature.

7. Prepare PNP solution
Each PNP tablet (ACC 00404) will make 5 ml of PNP solution, at a concentration of 1 mg/ml, about enough for five 8-well strips.

About 15 minutes before the end of the above incubation step, measure 5 ml of room temperature 1X PNP buffer for each tablet you will be using. Then, without touching the tablets, add the PNP tablets to the buffer.

Note: Do not touch the PNP tablets or expose the PNP solution to strong light. Light or contamination could cause background color in negative wells.
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8. Wash plate
As before, wash the plate 8 times with 1X PBST.
Inspect the wells looking for the presence of air bubbles. Tap firmly on the paper towel to remove remaining wash buffer and any air bubbles. If air bubbles are still present they may be broken with a clean pipette tip.

9. Add PNP substrate solution
Dispense 100 µl of PNP substrate into each testwell.

10. Incubate plate
Cover plates and incubate for 60 minutes. Plates should be protected from direct or intense light.

11. Evaluate results
Examine the wells by eye, or measure on a plate reader at 405 nm. Air bubbles which are present at the time of reading can alter results, if in the light path. Agdia recommends that bubbles be eliminated prior to reading.
Wells in which color develops indicate positive results. Wells in which there is no significant color development indicate negative result. Test results are valid only if positive control wells give a positive result and buffer wells remain colorless.
Results may be interpreted after more than 60 minutes of incubation as long as negative wells remain virtually clear.

Buffer Formulations
Carbonate Coating buffer (1X)
Dissolve in distilled water to 1000 ml:
- Sodium carbonate (anhydrous) 1.59 g
- Sodium bicarbonate 2.93 g
- Sodium azide 0.2 g
Adjust pH to 9.6. Store at 4° C.

PBST Buffer (Wash Buffer) (1X)
Dissolve in distilled water to 1000 ml:
- Sodium chloride 8.0 g
- Sodium phosphate, dibasic (anhydrous) 1.15 g
- Potassium phosphate, monobasic (anhydrous) 0.2 g
- Potassium chloride 0.2 g
- Tween-20 0.5 g
Adjust pH to 7.4

PNP Buffer (1X)
Dissolve in 800 ml distilled water:
- Magnesium chloride hexahydrate 0.1 g
- Sodium azide 0.2 g
- Diethanolamine 97.0 ml
Adjust pH to 9.8 with hydrochloric acid. Adjust final volume to 1000 ml with distilled water. Store at 4° C.
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