

Plum pox virus (PPV) Reagent Set

ELISA for the detection of PPV, alkaline phosphatase label
Catalog number: SRA 31502

List of contents

Lot number	Item	500 wells	1000 wells	5000 wells
_____	Coating antibody	0.275 ml	0.525 ml	2.525 ml
_____	Alkaline phosphatase enzyme conjugate	0.275 ml	0.525 ml	2.525 ml
_____	Positive control	1	1	5
_____	RUB3 enzyme conjugate diluent	55 ml	110 ml	550 ml
	The above items should be stored at 4° C.			
_____	General extract buffer 4 (GEB4)	82.5 g	165 g	5 x 165 g
_____	Tween 20	2 x 30 ml	4 x 30 ml	2 x 250 ml
_____	96-well microtiter plates	5	10	50
	The above items should be stored at room temperature.			

Materials required but not provided

- Distilled water
- Coating buffer, wash buffer and PNP substrate and tablets - see page 6 for formulations or purchase an Agdia buffer pack (ACC 00113)
- Paper towels
- Micropipette
- Micropipette tips
- Grinding device
 - Sample extract bag (catalog number ACC 00930) and tissue homogenizer (catalog number ACC 00900) or
 - mortar and pestle
- Airtight container for incubations

Optional materials

- Postcoat 10 buffer – this buffer extends the life of coated plates by 8 weeks. This buffer is available from Agdia (catalog number ACC 00650).

Storing the kit

Store all kit components at the recommended temperature to assure their full shelf life. Do not store prepared 1X buffers from day to day.

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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.

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-M, PPV-D, PPV-EA and PPV-W. This test does not recognize PPV-C (cherry isolate).

Test Principle

The test system for PPV is a direct DAS ELISA. Antibodies specific to PPV are coated to the testwells of a microtiter plate. Samples are added to the microtiter plate. If PPV is present in the sample, it is bound by the antibodies and captured on the microtiter plate. After sample incubation, the plate is washed to remove any unbound sample. A polyclonal antibody conjugated to alkaline phosphatase is added and binds to any captured PPV. After a brief incubation the plate is washed to remove any unbound conjugate. PNP substrate is added to the microtiter plate. If the alkaline phosphatase conjugate is present a color will be produced signifying the presence of PPV. The color reactions can be measured with a spectrophotometer or observed visually.

Limitations

The following is a description of factors that could limit test performance or interfere with proper test results.

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.

Sample Dilution: ELISA performance is very dependent on the proper sample 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.

References

1 Knapp, E., Machado. A.C., Puhringer, H., Wang. Q., Hanzer, V. Weiss, H., Weiss, B., Katinger, H., Machado, M.L.C. 1995. Localization of fruit tree viruses by immuno-tissue printing in infected shoots of *Malus* sp. And *Prunus* sp. J. Virol. Meth. 55:157-173.

2 Sheneder, W.L., Fredrick, R.D., and Damsteegt, V. D. 2003. New test on tap for tracking plum pox. Agric. Res/ Oct 2003, P.11.

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Coat testwells of ELISA plate

1. Prepare humid box

Prepare a humid box by lining an airtight container with a wet paper towel. Keeping the ELISA plates in a humid box during incubation will help prevent samples from evaporating.

2. Prepare coating antibody

Note: Coating antibody should be prepared in a container made of a material such as polyethylene or glass that does not readily bind coating antibody.

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

Prepare the volume of coating buffer (formulation on page 6) needed for the test. You will need 100 µl of coating antibody solution for each testwell you are using, therefore a 96 testwell plate requires 10 ml of coating buffer.

After the coating buffer is measured, add the appropriate volume of concentrated coating antibody to the coating buffer at the dilution given on the label. Use a new, sterile pipette tip to prevent contamination of the concentrated coating antibody.

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

Mix the prepared coating antibody thoroughly and use immediately.

3. Coat plate

Pipette 100 µl of the prepared coating 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).

5. Wash plate

Empty the wells into a sink or waste container. Fill the testwells to overflowing with 1X PBST, then quickly empty them again. Repeat 2 more 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 which is available separately from Agdia. (Catalog number ACC 00650).

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General extract buffer 4 (GEB4) GEB4 is used to dilute and extract samples. Refer to the sample extraction guidelines below for appropriate sample to buffer ratios.

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

To make 1L 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.

GEB4 can be stored in the refrigerator, (4° C to 6° C) for up to 3 months if handled properly. Agdia recommends preparing only as much buffer as is needed for one day.

This buffer contains 0.02% sodium azide.

Sample extraction

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

Samples can be ground in Agdia sample mesh bags with a tissue homogenizer. This method is especially useful for processing large numbers of samples. You can also use a mortar and pestle, or other grinding device to grind samples. If using a mortar and pestle, wash and rinse it thoroughly between each sample.

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.

100 µl of the ground and diluted sample will be added to the testwell.

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 sample extract buffer into the buffer wells.

2. Incubate plate

Set the testwells inside the humid box and incubate for 2 hours at room temperature or overnight in the refrigerator (4°C) for 14-16 hours.

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3. Prepare enzyme conjugate

Note: Always prepare enzyme conjugate within 10 minutes before use.

The alkaline phosphatase enzyme conjugate is concentrated and must be diluted with RUB3 enzyme conjugate diluent before use. The recommended conjugate to diluent ratio is given on the label.

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. Use a new, sterile pipette tip to prevent contamination of the enzyme conjugate.

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

Mix the enzyme conjugate thoroughly.

4. Wash plate

When the first incubation is complete empty the testwells into a sink or waste container in a manner that does not allow contents of one testwell from mixing with another testwell.

Then, fill all the testwells to overflowing with 1X PBST (formulation on page 6), and quickly empty. **Repeat this PBST wash step 6 to 8 times.** It is very important that all testwells are thoroughly washed.

After washing, hold the plate upside down and tap firmly on a paper towel to remove excess liquid.

Quickly inspect the testwells. All wells should be free of plant tissue or air bubbles. If tissue or bubbles are present repeat the washing and tap firmly on a paper towel.

5. Add enzyme conjugate

Dispense 100 µl of prepared enzyme conjugate to each testwell.

6. Incubate plate

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

7. Wash plate

As before, wash the plate 4 to 8 times with 1X PBST.

Quickly inspect the wells looking for the presence of air bubbles. Repeat the washing and tap firmly on the paper towel to remove air bubbles.

8. Add PNP substrate solution

Add 100 µl of PNP substrate solution to each testwell.

9. Incubate plate

Incubate the plate for 60 minutes. Keep testwells away from strong light.

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10. Measure color

Measure the optical density of the testwells on a plate reader at 405 nm. Read plates again after 120 minutes. Wells in which color develops 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 buffer wells remain colorless.

Technical service

If you have any questions about using this kit, please contact Agdia, Inc. Monday – Friday by phone (574-264-2014 or 1-800-622-4342) or by email (info@agdia.com).

Buffer formulations

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 substrate (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. About 15 minutes before the substrate step, measure the volume of 1X PNP buffer needed. Then, without touching the tablets (5 mg each), add one PNP tablet for every 5 ml of 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.

Date _____ Test _____

Test performed by _____

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

