

Product Information: AgriStrip Plum pox virus (PPV; Sharka)

PPV AgriStrip - a rapid assay for the detection of Plum pox virus (PPV)

Intended use

The rapid assay PPV AgriStrip is produced by BIOREBA for identification of PPV (Sharka) in *Prunus* sp. showing symptoms such as chlorotic spots, blotches, bands, rings or line patterns on leaves (Fig. 3,4). Later symptoms include uneven ripening, blotching and rings on fruits (Fig. 1). The concentration of PPV in tissues of fruit trees may vary considerably. For example, in peach and abricot trees, the concentration varies even within the same leaf.

Fig. 1. Sharka symptoms on fruit



These antibodies have been truly validated with extensive collections of virus isolates from over 20 different countries; e.g. at Palacky University in Olomouc as well as in independent studies „ringtests“, carried out at IVIA, Valencia, Spain (COST 88 PPV workshop) and at the Virological Laboratory Gödöllő, Hungary, where all isolates from different host and geographic origins were detected.

The sensitivity attained with these antibodies in the AgriStrip lateral flow is very similar to the sensitivity in the DAS-ELISA format (less than 2-fold difference in dilution series up to 1:640, w/v). In parallel tests with field samples of apricot, peach and plum, the accordance between the two test formats was 100% so far.

Test principle

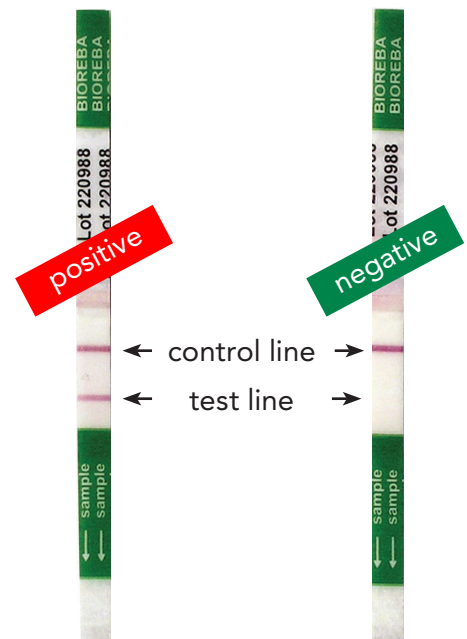
The rapid assay PPV AgriStrip is a lateral-flow immunochromatographic test based on an antigen-antibody reaction that is initiated by inserting the strip into the sample extract. The sample liquid migrates upwards and red colored lines will become visible within a few minutes.

Both test and control lines become visible with positive extracts (containing PPV), whereas negative samples produce only the upper control line (Fig. 2). Intense coloration is reached within 10 – 15 min and the result can be registered.

Dried test strips can be kept as permanent records.

Please read the notes on page 2-3 before starting the assay.

Fig. 2 Test strip



Specificity and sensitivity

The antibodies used for this AgriStrip assay were made against an isolate of PPV from the Netherlands and are detecting the «full spectrum» of PPV isolates, i.e., D, M, EA, C (sour and sweet cherry) and W strains.

Assay procedure

Sampling and extraction

Best tissues for sampling are blossoms or symptomatic leaves and their petioles of suspicious *Prunus* species in early summer. With leaves of peach and apricot showing symptoms predominantly at one half, it is crucial to sample symptomatic tissue (Fig. 4).

Place a piece of **2.5 x 3 cm of leaf** (that is approx. **0.15 g**) or three blossoms in an extraction bag, then add 3 ml of extraction buffer B with a disposable pipette* (Fig. 5a). Grind the tissue with the handheld homogenizer on a flat surface with **few movements for not more than 3-4 seconds** to obtain an 1:20 (w/v) extract (Fig. 5b).

With a disposable pipette* first add 2 drops of extraction buffer into a cuvette, followed by 2 drops of the extracts resulting in a total of 150 µl of the optimal dilution of 1:40 (w/v) see Fig. 5c and Fig. 6.

Test

Place a strip with the end marked «sample» into the extract (Fig. 5d). Observe the coloration of the lines that is completed after 15 min and can then be evaluated. Avoid any wetting of the strip above the sample mark with plant extract.

To prevent any confusion, label extraction bags, cuvettes and strips with a permanent marker.

Analysis of results

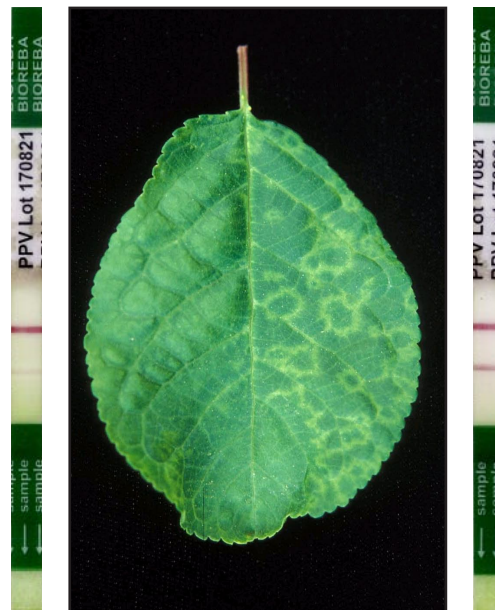
- 1) Both, a clearly visible test and control line are obtained with extracts containing PPV as shown in Fig. 2. This means the test is **positive**.
- 2) The test is **negative**, if the control line but no test line appears after 15 min. This means that no PPV is in the extract or in a concentration below the detection limit.
- 3) If neither test nor control line become visible, the test is **invalid** and should be repeated with a fresh strip.
- 4) Rarely, a faint test line might become visible after 15 – 30 min. Repeat the test in this case with a new extract or send the sample to a laboratory for verification with another method, such as RT-PCR.

Dried test strips can be stored as permanent record, even though the coloration of bands is stronger when the strips are still wet.

Fig. 3. Sharka symptoms on plum leaves



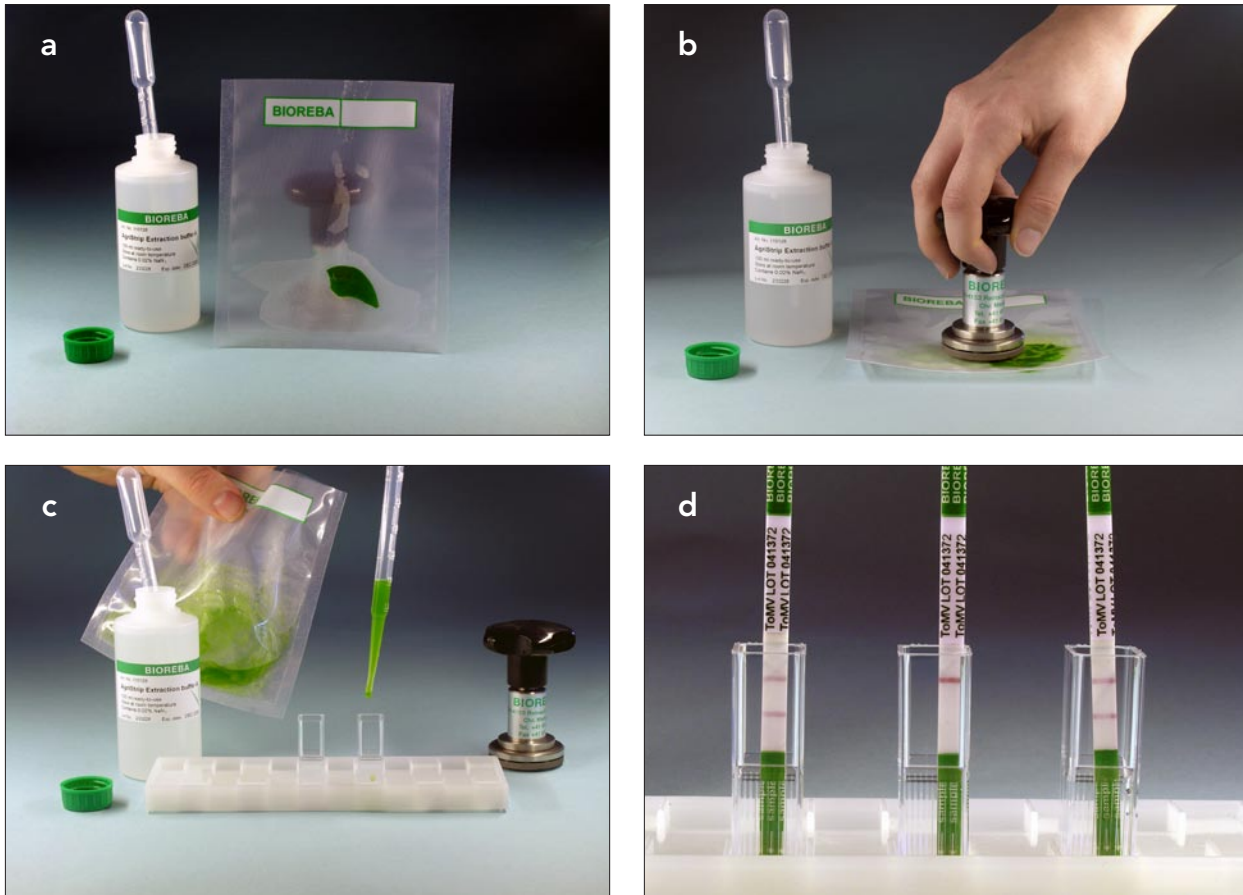
Fig. 4. Inhomogeneous distribution of PPV



Notes

- 1) Store the strips and the extraction buffer at 4°C. However, exposure to ambient temperature (10 – 30°C), such as during transport and use in the field, does not affect the quality. Keep the packaging (containing desiccant bags) always hermetically closed. Absorbed moisture by the strips can lead to poor results or even complete failure of the test.
- 2) Strips must be used before the expiration date indicated on the label of the packaging.
- 3) Use the PPV AgriStrip always with the Extraction buffer B supplied with the strips. Other buffers might fail.
- 4) It is recommended to run the test at 15 – 25°C. At temperatures below 15°C, the speed of color development of test and control lines slows down and the test can be evaluated only after 20 – 40 min.

Fig. 5. AgriStrip assay procedure



5) The extraction buffer contains sodium azide as preservative. Keep out of reach of children and do not ingest.

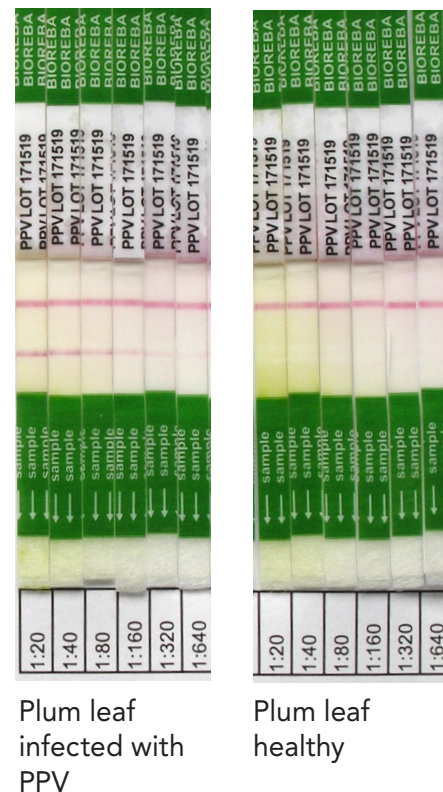
Hygiene measures

To avoid spread of the pathogen, all disposables (extraction bags, pipettes, and cuvettes) must be properly disinfected. This can be done by submerging for at least 60 min in a freshly prepared 0.1 % bleach solution (sodium hypochlorite). After that, this material can be disposed of according to local regulations. Please contact local phytosanitary authorities for specific recommendations.

* Use of the disposable pipettes

Do not accidentally contaminate the extraction buffer. Please make sure that the disposable pipettes are immediately disinfected and disposed of, after having been in contact with samples. Use always a fresh pipette from the pouch for dosing extraction buffer.

Fig. 6. Sensitivity of lateral flow immunochromatography.



PPV (Sharka) AgriStrip



Fig. 4. PPV AgriStrip Complete kit (Art. No. 150581)

Content of the Complete kit

- 25 strips packed with desiccant bags
- 100 ml **AgriStrip** Extraction buffer B
- 25 disposable pipettes
- 25 extraction bags
- 25 disposable cuvettes
- 1 cuvette rack
- Note: the homogenizer hand model is optional and not included in the Complete kit.

Ordering information

Product	Art. No.	Assays
PPV AgriStrip Complete kit	150581	25
PPV AgriStrip Single strips*	150582	25
PPV AgriStrip Single strips*	150583	100

* including ready-to-use **AgriStrip** Extraction buffer B

Optional products	Art. No.	Size
Cuvettes, disposable	2534	100
Cuvette rack, holds 12 cuvettes	2166	1
Pipettes, disposable	2292	500
Extraction bags «Universal»	430100a	100
Homogenizer hand model	400010	1

Disclaimer

This BIOREBA product is guaranteed to meet the specifications mentioned in this product information. No further warranties are given. Should this product fail for reasons other than inappropriate handling or misuse, BIOREBA AG will replace the product free of charge or refund the purchase price.

BIOREBA AG shall not be liable for any direct or indirect, special or consequential damages of any kind resulting from the use of this product.