

Product Information: AgriStrip *Erwinia amylovora* (Ea)

Ea AgriStrip - a rapid assay for the detection of *Erwinia amylovora* (Ea) and diagnosis of fire blight

Intended use

The rapid assay Ea AgriStrip for *Erwinia amylovora* detection is produced by BIOREBA and has been validated in cooperation with the Swiss federal research station Agroscope Changins-Wädenswil (ACW). This project was supported by the Innovation Promotion Agency (CTI) of the Swiss government. The rapid assay enables confirmation of the presence of fire blight pathogen on-site and within minutes in pome fruit and related ornamentals and wild plants. Samples are best taken in the transition zone between healthy and symptomatic tissues containing the highest concentration of bacteria (Fig. 2a). Active cankers and bacterial ooze are also suitable for testing (Fig. 2b).

Asymptomatic flowers, inactive cankers, and roots, however, may not give reliable results due to low bacterial concentration.

Although harmless for human, this bacterium is regarded as a quarantine organism because of its high infection risk for other host plants. Appropriate hygiene/disposal after sampling is critical to prevent spread of this devastating disease, and local phytosanitary regulations must be followed.

Test principle

The rapid assay Ea AgriStrip is a lateral-flow immunographic 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 *Erwinia amylovora*), whereas negative samples produce only the upper control line (Fig. 1). Intense coloration is reached within 10 – 15 min. and the result can be registered.

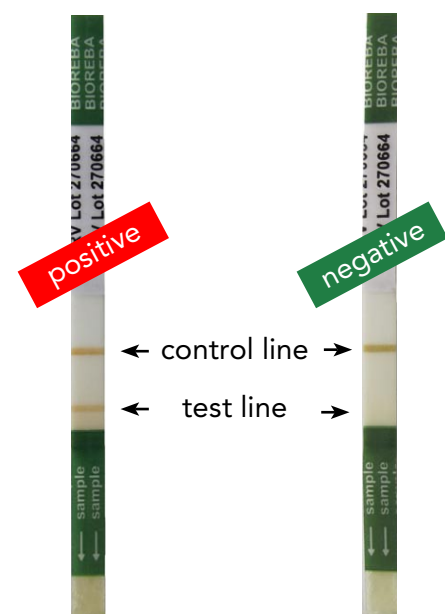
Dried test strips can be kept as permanent records.

Specificity and sensitivity

Specific antibodies used in this rapid assay Ea AgriStrip were produced against a mixture of different isolates of *Erwinia amylovora* from Europe and the USA and react only with the fire blight pathogen. The detection limit of the rapid assay is between 5×10^5 and 10^6 cfu/ml. Positive reactions occur also with the related fire blight pathogen found only in Asia (*Erwinia pyrifoliae*). In contrast, no cross-reaction was observed with *Pseudomonas* sp., *Pantoea agglomerans*, or other bacteria commonly found in pome fruit samples.

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

Fig. 1
Test strip



Test procedure

Sampling on symptomatic wood

Remove the bark layer and scrape with a sharp and disinfected knife small longitudinal pieces of approx. 1 mm thickness at the transition zone between healthy and symptomatic cambium tissue (Fig. 2a). First transfer 4 ml of extraction buffer with a disposable pipette* in an extraction bag, then add approx. 0.2 – 0.5 g (5 - 10 pieces) of the cambium material (Fig. 3a). Crush the tissue (e.g. with a rubber hammer) thoroughly on a flat surface to mix the extruded plant sap with the extraction buffer (Fig. 3b). Then transfer 4 drops (approx. 150 µl) of this extract into a cuvette with a disposable pipette* (Fig. 3c).

Sampling of bacterial ooze

First add 4 drops of extraction buffer in a cuvette. Then take a tiny amount of the bacterial exudation (Fig. 2b) by touching with the tip of the disposable pipette* and suspend it in the extraction buffer in the cuvette. Do not take more of the ooze than what is necessary to get a slight turbidity of the buffer. A highly excessive bacterial suspension (opaque solution) can interfere with test efficacy.

Test

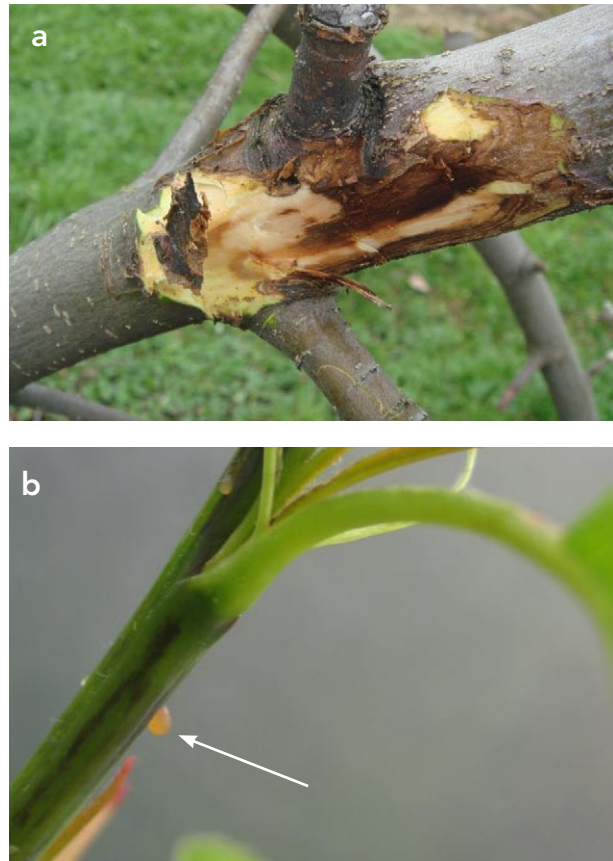
Place a strip with the end marked «sample» into the extract (Fig. 3d). Observe the coloration of the lines that is completed after 15 min. and can then be evaluated. Avoid any wetting of the strip above the sample mark with plant extract.

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

Analysis of results

- 1) Both, a strong test and control line are obtained with extracts containing high number of bacteria ($> 10^7$ cfu/ml), as shown in Fig.1. This means the test is **positive**.
- 2) A test line which appears weaker than the control line is obtained with extracts containing low numbers of bacteria (5×10^5 to 10^6 cfu/ml). This means the test is **positive**.
- 3) The test is **negative**, if the control line but no test line appears after 15 min. This means that no bacteria are in the extract or in a number below the detection limit.

Fig. 2. Sampling



4) If neither test nor control line become visible, the test is **invalid** and should be repeated with a fresh strip.

5) 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.

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

* 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. 3. AgriStrip Test procedure



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 of the strips (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 Ea AgriStrip always with the Extraction buffer B supplied with the strips. Other buffers might fail.
- 4) It is recommended to run the test at ambient temperature (15 – 25°C). At temperatures below 15°C, the speed of color development of test and control lines slows down.
- 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 0.1 % bleach solution (sodium hypochlorite) prepared freshly every day. After that, this material can be disposed of together with the wooden residues of the samples according to local regulations. Please contact local phytosanitary authorities for specific recommendations.

Ea AgriStrip



Fig. Ea AgriStrip Complete kit
(Art. No. 153081)

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 rubber hammer is not included in the Complete kit.

Ordering Information

Product	Art. No.	Assays
Ea AgriStrip Complete kit	153081	25
Ea AgriStrip Single strips*	153082	25
Ea AgriStrip Single strips*	153083	25

* incl. **AgriStrip** Extracton buffer B, ready-to-use

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

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.