

Xac ImmunoStrip® Test

ImmunoStrip® test for detection of *Xanthomonas axonopodis* pv. *citri* the causal agent of Asiatic Citrus Canker

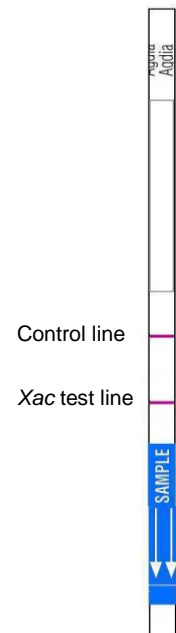
Catalog no. STX 92200

CONTENTS	STX /0025	ISK /0005	ISK /0025
ImmunoStrip®	25 strips	5 strip	25 strips
Sample bag with SEB1 buffer*	not included	5 bags	25 bags
Instruction	1	1	1

The Xac ImmunoStrip® test must be used with **SEB1** sample extraction buffer.

YOU WILL NEED

- Scissors, a pen, and a knife or razor blade
- **SEB1** sample extraction buffer, available as:
 - Mesh sample bag containing SEB1 buffer (ACC 00936)
 - SEB1 powdered buffer (ACC 00996)
- Sample bag holder –a small letter holder can be used.



STORAGE

Keep the strips tightly sealed in the container with the desiccant at all times. Store the strip container and sample extraction buffer in the refrigerator (4°C/39.2°F) or in a cooler between uses. Allow the strip container and sample extraction buffer to warm up to room temperature prior to use.

SAFETY

Sample extraction buffer and strip tests are non-hazardous

INTENDED USE

This test detects the bacteria *Xanthomonas axonopodis* pv. *citri*, A-strain, the causal agent of Asiatic citrus canker. The Xac ImmunoStrip® test is intended to be used as a confirmation tool to support the visual diagnosis of Asiatic citrus canker on fruit, or on leaves and stems of mature citrus trees. In verification studies, this test had a 97% confirmation rate to visual diagnosis of fruit lesions and a 100% accuracy rate with known cultures of A-strain Xac.

This test has a limitation when testing leaf lesions of seedlings in the nursery or of young trees growing in the field less than two years. In verification studies this test also detected 43% of cultures of *X. axonopodis* pv. *citrumelo* which causes citrus bacterial spot (CBS) lesions on citrus seedlings grown in the nursery. CBS lesions may be present up to two years on young trees after out-planting in the field. Other methods are needed to make a diagnosis under such a circumstance.

PLANT SAMPLES

When taking a sample it is best to take an entire lesion and a small amount of surrounding tissue. “The earliest symptoms on leaves appear as slightly raised tiny blister-like lesions” “As lesions age, they turn tan to brown, and a water-soaked margin appears surrounded by a chlorotic halo.” “The center of the lesion becomes raised and corky”¹ after time the center of leaf lesions will be more like a pit or crater.²

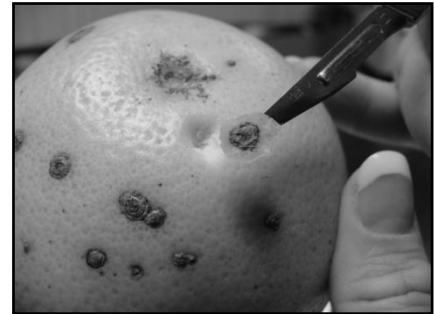
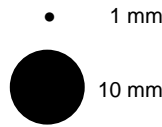
“On twigs and fruit, citrus canker symptoms are similar...” However, “no chlorosis typically surrounds twig lesions, but may be present on fruit lesions. Chlorosis symptoms can fade over time.”¹

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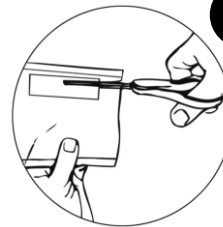
Sample Preparation

1 Plant samples:
Samples should consist of a single lesion between 1 mm and 10 mm in size. The lesion needs to be ground in buffer. The Xac ImmunoStrip® must be used with SEB1 Sample Extraction Buffer. Do not use any other sample extraction buffer. This instruction describes the use of Agdia mesh sample bags, though other means of extracting samples may be preferred.



Note: If you are using a knife or razor blade to cut samples, disinfect the cutting area and the knife or razor blade with alcohol between each sample.

2 Cut off the top of the mesh sample bag, being careful not to spill the buffer.



3 Place the sample pieces between the mesh linings of the mesh sample bag.



4 Rub the bag with a pen or blunt object to completely crush and mix the sample. Use only one sample per bag, and be sure to label each bag.

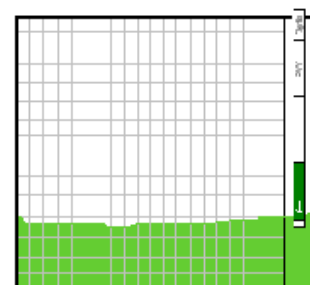


Instructions for use

5 Remove the Xac ImmunoStrip® from the container. When handling the strips, always grasp the top of the strip marked with the test name. Do not remove the protective covering.

Insert the end of the ImmunoStrip® marked "sample" into the pouch, as illustrated. Allow the strip to run for a maximum of 30 minutes or until both the control line and test line appear.

Note: Do not allow much more than 0.5 cm or ¼ inch of the end of the strip to be submerged in the extract. The end of the strip should remain in the extract during the test.



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Results

The **control line** assures that the test is working properly. If the control line does not appear, the test is invalid.

If the sample is **positive (+)** for Xac, the **test line** will appear. The test line will be red to purple in color just as the control line. The color intensity of the test line will vary. A positive result can appear and be interpreted in less than 30 minutes.

If the sample is **negative (-)** for Xac, the **test line** will not appear. Samples with low bacterial titer may not be detected with the ImmunoStrip®. Allow the strip to run the full 30 minutes before ruling the sample negative.

Use the images to the right as a guide to determine results. If necessary, align the ImmunoStrip® with the images to determine the exact positions of the test line and control line.

Saving and confirming test results

If you wish to keep a strip as permanent record, cut off the sample pad (blue end marked "sample"). This prevents any liquid still in the sample pad from interfering with test lines. Then blot the ImmunoStrips and sample pads between paper towels. The strips can be scanned or taped to a piece of paper.

If you wish to confirm positive results keep the sample pad. This sample pad can be sent to a lab and analyzed by PCR. Positive results can be confirmed from the remaining sample still in the sample pad. Allow the sample pad to dry and then place the pad and a label identifying the test sample in a plastic bag. Avoid handling the sample pad with bare fingers as much as possible. Contaminants from the hands could degrade the sample. Contact Agdia Testing Services at 1-574-264-2014 or info@agdia.com for more information on PCR testing.

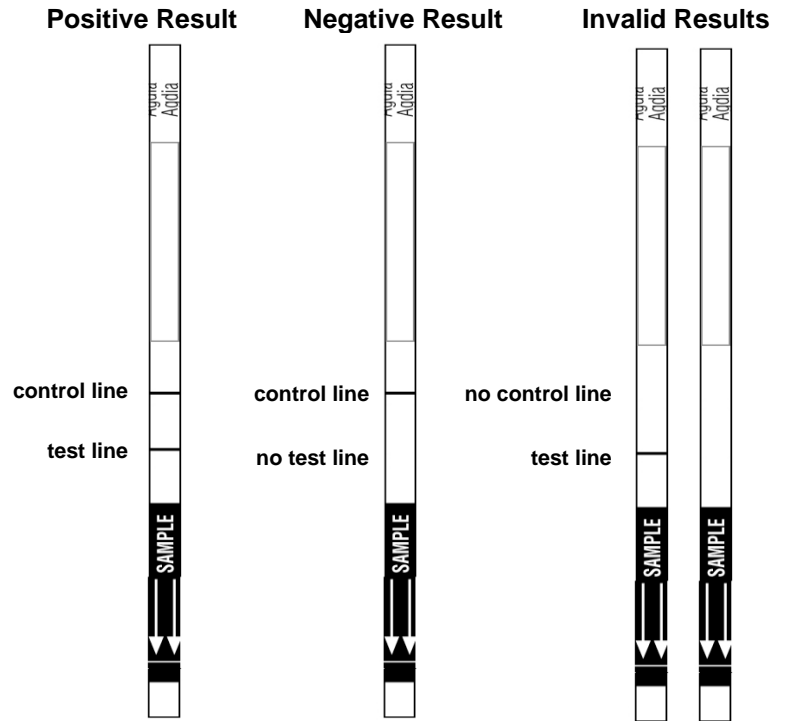
Limitations

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

Sample Extraction Buffer: The Xac ImmunoStrip® must be used with SEB1 Sample Extraction Buffer. Do not use any other sample extraction buffer.

Sample Dilution: Strips will not properly absorb sample extracts containing large amounts of tissue. Use only single lesions between 1 to 10 mm in size.

Submerging the Strip: Test strips must not be submerged more than 0.5 cm or ¼ inch. If too much of the strip is submerged, certain components of the strip are released into the sample instead of being wicked upward by the strip. This most often results in a failed test in which no control line forms.



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Limitations (cont.)

Expiration: Test should be used within one year of purchase.

Storage: Test results may be weak or the test may fail if the storage instructions are not followed properly. **The ImmunoStrip® package must remain sealed with desiccant when not in use to prevent moisture degradation.** Moisture degradation will affect test results.

Results: Some plant tissues may cause what appears to be a green test line. This may be due to the tissue type or to samples containing too much tissue. Samples producing such a result should be diluted further and retested. If the green line persists, contact Agdia directly for further assistance.

Cross-reactivity: This is a test for the common Asian strain *Xanthomonas axonopodis* pv. *citri* the causal agent of the Asiatic type of citrus canker. This test does not detect the A strain variants of A^w, A*, A-Etrog which have very narrow host ranges. Cross-reactivity testing with isolates of *Xanthomonas*, *Psuedomonas*, and other bacteria causing citrus leaf spot and citrus canker was performed showing some cross reaction with isolates of *Xanthomonas axonopodis* pv. *citrumelo*, which only infects leaves of citrus seedlings in the nursery. This cross-reactivity limits the use of the Xac ImmunoStrip® in testing nursery plants or young trees planted in the field less than two years.

Sensitivity: Xac may be present in low concentrations or may be unevenly distributed on the plant. It is important to take samples from tissue showing symptoms to improve your ability to detect the bacteria. It is best to test lesions.

Reference

1. Schubert, T. S. and Sun, X. (2003) Bacterial Citrus Canker. Plant Pathology Circular of Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL. No. 377.
2. Graham, J. H., Gottwald, T. R., Cubero, J., and Achor, D. S. (2004) *Xanthomonas axonopodis* pv. *citri*: factors affecting successful eradication of citrus canker. *Molecular Plant Pathology*, 5 (1), 1-15.

Technical Assistance

For technical assistance or questions regarding the use of this test system, please contact Agdia, Inc. Monday-Friday by phone at 1-800-622-4342, 1-574-264-2014 or by email at info@agdia.com.