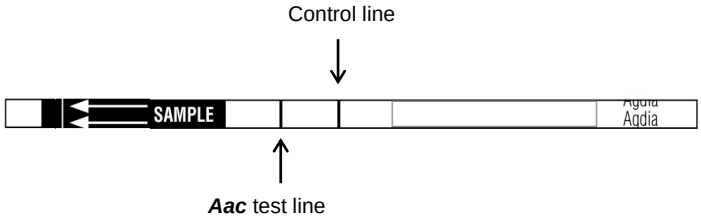
Aac ImmunoStrip® Test

ImmunoStrip test for detection of *Acidovorax avenae* subsp. *citrulli*
Catalog number: STX 14800

CONTENTS	STX /0025	ISK /0005	ISK /0025
ImmunoStrip, blue label	25 strips	5 strips	25 strips
Sample bag containing **SEB4** buffer*	not included	5 bags	25 bags
Instruction	1	1	1

*The *Aac* ImmunoStrip test must be used with **SEB4** buffer.



YOU WILL NEED

- Scissors, a pen, and a knife or razor blade
- **SEB4** sample extraction buffer, available as:
  - Mesh sample bag containing SEB4 buffer (ACC 00958)
  - SEB4 powder, 5.7 grams makes 1 liter (ACC 01958)
- Letter holder or other device to hold sample extraction bags
- 1.5 mL microcentrifuge tube (for testing culture samples)
- Microcentrifuge tube rack (for testing culture samples)
- Transfer pipette (for testing culture samples)

STORAGE

Keep the strips tightly sealed in the container with the desiccant at all times. Store the container in the refrigerator (4 °C) between uses. The sample buffer should also be refrigerated (4 °C) when not in use. Allow the bottle to warm up to room temperature after removal from the refrigerator before opening.

INTENDED USE

This test is a rapid and reliable screening tool for *Acidovorax avenae* subsp. *citrulli* (*Aac*), the causal agent of bacterial fruit blotch in watermelon, squash, pumpkins, and cantaloupe. The *Aac* ImmunoStrip is intended for use with leaves, fruit, and seedlings exhibiting symptoms of *Aac*. Bacterial cultures may also be tested. The *Aac* ImmunoStrip must be used with SEB4 extraction buffer. Do not use any other sample extraction buffer.

Sample Preparation and Test Procedure

This instruction describes the use of Agdia mesh sample extraction bags for grinding tissue samples, although other means of extracting samples are acceptable. Each bag contains 3 mL of SEB4 sample extraction buffer.

1

Seedling samples:

Symptoms of watermelon fruit blotch begin on seedlings as small, water-soaked lesions (Figure 1) on the underside of the cotyledons or leaves; lesions later become necrotic (Figure 2). Samples should be taken locally from leaves or cotyledons of seedlings showing signs of infection during grow-out. Using a knife or razor blade, cut around the symptomatic region. Samples should be ground in SEB4 buffer at a 1:20 w/v ratio. Agdia sample extraction bags would require about 0.15 g of tissue.

Figure 1:

Plant samples:

Disease symptoms on foliage can progress throughout the growing season and may not be particularly obvious or may be confused with other diseases. Leaf lesions are generally light brown to reddish brown and will often spread along the midrib of the infected leaf. Samples should be taken from leaves or stems (Figure 2) of plants showing water-soaked lesions or necrotic (brown) spots and ground in SEB4 buffer at a 1:20 w/v ratio. Agdia sample extraction bags would require about 0.15 g of tissue.

Figure 2:

Fruit samples:

Early infection
Symptoms on susceptible fruit begin as small water-soaked areas (a few millimeters in diameter) and rapidly expand into larger lesions with irregular margins. The entire surface of the fruit may become covered with these dark green lesions within a few days. Samples should be taken locally from the infected area (Figure 3) of the fruit by cutting a wedge just deep enough to get past rotten material. The tip of this fruit wedge should be ground in SEB4 buffer at a 1:10 w/v ratio. Agdia sample extraction bags would require about 0.3 g of tissue.

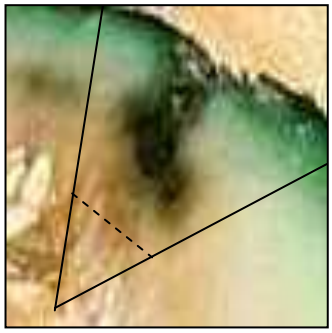


Figure 3:

Late infection
Older fruit lesions become necrotic and may crack, exposing the interior of the rind and the flesh of the melon. A whitish bacterial ooze may exude from the splits, and eventually, infected fruit will rot. Samples should be scraped locally from the infected rind of fruit (Figure 4) showing necrotic lesions and ground in SEB4 buffer at a 1:5 w/v ratio. Agdia sample extraction bags would require about 0.6 g of tissue.

Figure 4:

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.

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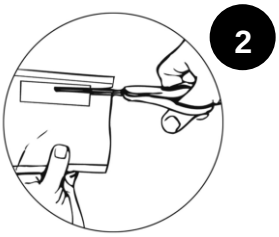
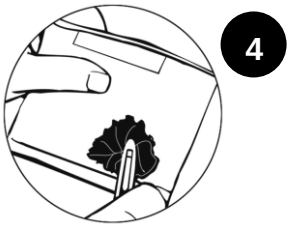
2 Cut open the SEB4 sample extraction bag along the top of the label. Be careful not to spill the buffer.



3 Insert the sample between the mesh linings near the bottom of the sample extraction bag.

4 Extract the sample by rubbing it gently between the mesh linings with a blunt object such as a pen or permanent marker.



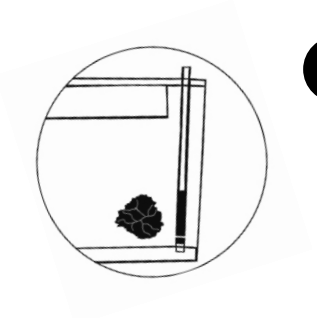
Culture samples:

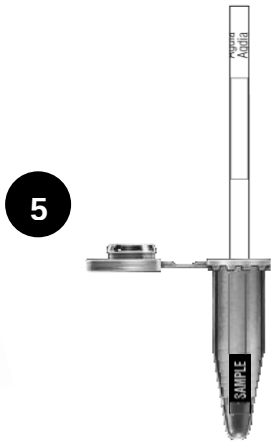
To test bacterial culture samples, use a toothpick to remove a bacterial colony from a culture plate. Stir into a tube containing 300 µL of SEB4 extraction buffer. Do not use *Aac* ImmunoStrips with cell culture broth. Let the samples sit in buffer for 1 minute, then use a transfer pipette to transfer 250 µL of the liquid to a 1.5 mL microcentrifuge tube.

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

Insert the end of the ImmunoStrip marked “sample” into the extract in the bag or tube. Place the bag in a letter holder or other device in upright position. If using a tube, place in a rack. Allow the strip to remain in the sample extract for 20-30 minutes.

Note: Do not allow the “sample” end of the strip to be submerged into the extract more than ¼” or to the white line on the ImmunoStrip label.





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Results

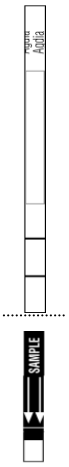
Maximum reaction occurs in 30 minutes, at which time the ImmunoStrip should be removed from the sample. Use the images to the right as a guide to determine results. If necessary, align the ImmunoStrip with the images to determine the exact positions of the test line and control line.

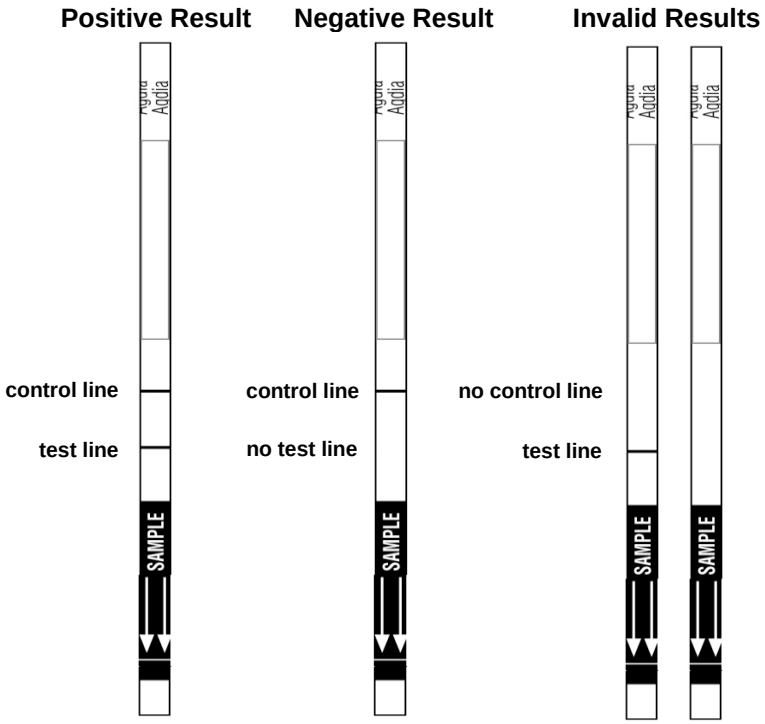
The **control line** assures that the test is working properly. If the control line does not appear, the test is invalid.

If the sample is positive (+) for *Acidovorax avenae* subsp. *citrulli*, 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.

If the sample is negative (-) for *Aac*, the **test line** will not appear. Samples with low levels of bacteria may not be detected with the ImmunoStrip.*

Note: If you wish to keep a strip as a permanent record, cut off the sample pad (green end marked "sample") and discard. This prevents any liquid still in the sample pad from interfering with results. Then blot the ImmunoStrips between paper towels.





Limitations

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

Sample Dilution: Strip performance is very dependent on the proper sample dilution (consult the recommended sample dilutions on page 2). Strips will not properly absorb sample extracts containing large amounts of tissue.

Submerging the Strip: Test strips must not be submerged more than 0.5 cm or ¼ inch into the extract. 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.

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

Storage: Test results may be weak or the test may fail if storage instructions are not followed properly. **The ImmunoStrip package must remain sealed with desiccant when not in use to prevent moisture degradation!** This may 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: The *Aac* ImmunoStrip has no known cross-reaction with other bacterial pathogens or A. *facilis*, a non-infectious pathogen, which produces similar symptoms to *Aac*.

***Sensitivity:** *Aac* may be present in low concentrations or may be unevenly distributed in the plant. It is important to take samples from tissue showing symptoms to improve your ability to detect the bacteria. The lower detection limit of this test is $4x10^5$ cfu/ml.

Technical Assistance

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