

Highlights:

- Results in only 5 minutes
- Simple protocol
- Highly specific for *Acidovorax avenae subsp. citrulli*

Contents of Kit:

- 25 QuickStix Strips packed in a moisture-resistant canister
- 25 Disposable Tissue Extractors (each consisting of a tube with punch cap and pestle)
- EB2 Extraction Buffer



Collect one leaf sample (include suspected spot)



Add 10 drops of buffer (0.5 mL per leaf snap)

Catalog Number AS 048 PT 25

Intended Use

The EnviroLogix QuickStix Kit for Bacterial Fruit Blotch (BFB) screens for the presence of *Acidovorax avenae subsp. citrulli* (Aac) that causes bacterial fruit blotch in cucurbits. The strip can detect the presence of bacteria at a very early stage, when it is difficult to differentiate BFB symptoms from other bacterial, viral or fungal infections and/or insect damage. The test detects bacterial extracellular polysaccharide (EPS) down to 10^5 to 10^6 cfu/mL.

How the Test Works

This test is for use on cotyledon or leaf tissue that is suspected to be infected with bacterial fruit blotch. It can also be used to test a bacterial colony (bacterial ooze) itself. For detection of Aac in fruit pulp and juice, call Tech Service for a customized application guide. To detect infected tissue or bacterial colonies with the EnviroLogix QuickStix Strip, samples must be extracted with the buffer provided. The antibody has been shown to detect all known Aac strains, and is very specific to Aac. It does not react with other *Acidovorax* spp or cucurbit pathogens including *Acidovorax facilis*, *Acidovorax avenae subsp. avenae*, *Acidovorax konjaci*, *Acidovorax cattleyae*, *Comomonas testosteroni*, *Burkholderia cepacia*, *Pseudomonas acidovorans*, and *Pseudomonas lachrymans*.

Each QuickStix Strip has an absorbent pad at both ends. The protective tape with the arrow indicates which end of the strip to insert into the extraction tube. The sample travels up the membrane strip and is absorbed into the larger pad at the top of the strip. The portion of the strip between the protective tape and the absorbent pad at the top of the strip is used to view the reactions as described under “Interpreting the Results”.

Sample Preparation

A. To extract leaf tissue:

Sampling Recommendation: Take a cotyledon or leaf sample that includes a suspect spot or area. The punch cap will result in a leaf sample of approximately 10 mm diameter, weighing about 0.03 grams.

1. Sandwich a section of cotyledon or leaf tissue between the cap and body of the Disposable Tissue Extractor tube. Snap **one** circular leaf punch by closing the cap. Push the leaf punch down into the tapered bottom of the tube with the pestle. Write the sample identification on the tube with a waterproof marker.
2. Holding the dropper bottle vertically, add 10 drops of buffer to the tissue sample (if using a pipette, add a total of 500 μ L of buffer). Insert the pestle into the tube and grind the tissue by rotating the pestle against the sides of the tube with twisting motions. Continue this process for 20 to 30 seconds, or until the leaf tissue is well ground. Remove and discard pestle.

B. To extract bacterial colony samples:

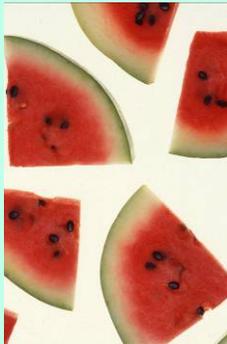
1. Add 10 drops of buffer to the Disposable Tissue Extractor tube (if using a pipette, add 500 μ L of buffer).
2. Using a sterile wooden toothpick or similar device, just touch the suspected colony. Swish the sample in the buffer, then discard toothpick.



Grind the tissue with pestle



Add a test strip, wait 5 minutes



How to Run the QuickStix Strip Test

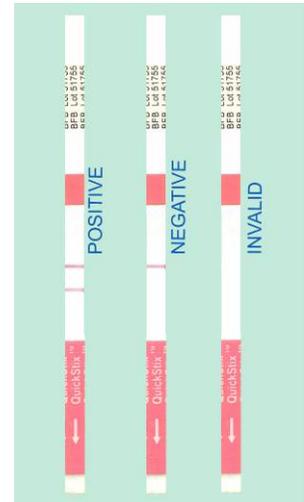
1. Allow refrigerated canisters to come to room temperature before opening. Remove the QuickStix Strips to be used. Avoid bending the strips. Reseal the canister immediately.
2. Place the strip into the extraction tube. The sample will travel up the strip. Use a rack to support multiple tubes if needed.
3. Allow the strip to develop for a full 5 minutes before making final assay interpretations. Positive sample results may appear much more quickly.
4. To retain the strip, cut off and discard the bottom section of the strip covered by the arrow tape.

Interpreting the Results

Development of the Control Line within 5 minutes indicates that the strip has functioned properly. Any strip that does not develop a Control Line should be discarded and the sample re-tested using another strip.

If the sample extract contains Bacterial Fruit Blotch infection, a second line (Test Line) will develop on the membrane strip between the Control Line and the protective arrow tape. The results should be interpreted as positive for *Acidovorax avenae* subsp. *citrulli*.

If no Test Line is observed after 5 minutes, the results should be interpreted as negative.



Kit Storage

This QuickStix Kit can be stored at room temperature, or refrigerated for a longer shelf life. Note the shelf life on the kit box for each storage temperature. The kit may be used in field applications; however, prolonged exposure to high temperatures may adversely affect the test results. Do not open the desiccated canister until ready to use the test strips.

Precautions and Notes

- This kit is designed for screening the presence or absence of Bacterial Fruit Blotch and is not meant to be quantitative.
- As with all tests, it is recommended that results be confirmed by an alternate method.
- The assay has been optimized to be used with the protocol provided in the kit. Deviation from this protocol may invalidate the results of the test.
- The test is recommended to be used on single leaf sample only. Compositing or pooling of samples is not recommended and may result in false negative.
- A negative result does not preclude the presence of bacterial fruit blotch infection in other areas or at other times.
- A strong positive result may safely be interpreted in as little as 2 minutes after sample addition. It is not safe, however, to conclude that a sample is negative before a full 5 minutes has elapsed. A weakly positive sample may require the full 5 minutes for a distinct Test Line to appear.
- Protect all components from hot or cold extremes of temperature when not in use. Do not leave in direct sunlight or in vehicle. A small portable cooler is recommended for field testing applications to protect the kit from extreme temperatures.



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