

**Intended Use**

This test is a rapid means of screening crops for *Ralstonia solanacearum* (*Rs*). The *Rs* ImmunoStrip is intended for use with plant samples exhibiting symptoms of *Rs*. The test can also be used to test bacterial culture samples. The *Rs* ImmunoStrip detects *Rs* to the species level and cannot differentiate race or biovar.

**Storage of Kit**

ImmunoStrips should be stored refrigerated (2 - 8 °C) between uses and tightly sealed in the desiccated container at all times.

ImmunoStrips and extraction buffer should be warmed to room temperature (18 - 30 °C) prior to use. You will notice the cold BEB1 extraction buffer will contain a white precipitate or appear cloudy. The BEB1 will become clear after warming which signals that the test components are ready to use.

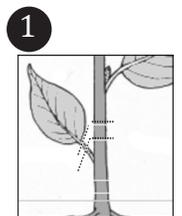
**Limitations**

This test is not recommended for testing the plant genus *Helleborus*, a member of the Ranunculaceae family. Internal data demonstrates that the *Rs* ImmunoStrip Test may produce non-specific reactions (false positives) when testing root and crown material of *Helleborus*.

Sensitivity: The lower detection limit of this test is 10<sup>4</sup> cfu/mL.

**PERFORMING THE ASSAY (\*Special Attention Required)**

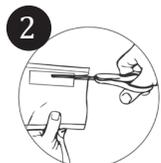
**Prepare Sample**



1 Samples should be taken from stems or petioles of plants showing signs of infection (wilting). Agdia sample extract bags contain 3 mL of extraction buffer, requiring 0.15 g tissue for the optimal 1:20 dilution. For most samples, an approximate sample size of 2.5 cm<sup>2</sup> or 1 inch<sup>2</sup> is adequate; however, thick or dense tissues can alter the targeted 1:20 dilution. Extraction and testing of overly degraded, dried, or large amounts of tissue can cause erroneous results.

When working with stems cut two cross section pieces at the first two internodes from the crown. The stem can be cut into smaller sections for easier grinding.

**Note: It is recommended that you use a clean cutting tool for each sample. If you must reuse the cutting tool, first wipe off the cutting edge and disinfect in a 10% bleach solution before cutting into a new sample.**



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

\*BEB1 Buffer is required to perform this assay.



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

**ImmunoStrip Kit (ISK) Includes**

- ImmunoStrips
- BEB1 sample extraction bags
- User guide

ImmunoStrips (STX) purchased separately do not include buffer filled mesh bags.

**What's required to perform the assay?**

- Scissors, knife or razor blade
- BEB1 sample extraction buffer
- Sample extraction equipment (e.g., Agdia sample extraction extraction bags; Agdia tissue homogenizer - ACC 00900 or blunt object such as a pen or marker)
- 1.5 mL microtubes, pipette tips and micropipette – when working with bacterial cultures
- Letter holder or other device to hold sample extraction bags



4 Extract the sample by thoroughly macerating it with an Agdia tissue homogenizer or a blunt object such as a pen or marker. An adequately extracted sample will result in a homogenous green or light brown colored solution. Allow the resulting solution to settle for 3 minutes before inserting the ImmunoStrip.

**Bacterial culture samples:** Use a toothpick to remove a colony of bacteria from a culture plate. Stir into a tube containing 300 µL of BEB1 extraction buffer. If using cell culture broth, the cell suspension should be at a concentration of 10<sup>6</sup> cfu/mL or above. Add 50 µL of the cell suspension to a tube containing 250 µL of BEB1 sample extraction buffer.

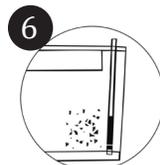
**Perform Assay**



5 Remove an ImmunoStrip then reclose 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 sample end of ImmunoStrip into the bag until submerged in the extract up to the white line. For best results, insert the ImmunoStrip into the channel portion of the bag (no mesh). Do not allow the side of the ImmunoStrip to come into contact with foam or bubbles (if present). Trimming the bag may also allow for more control when inserting the ImmunoStrip into the bag.

**\*Be sure to insert the "sample" end of the strip no more than ¼ inch or to the white line on the ImmunoStrip label.**



6 Place the bag in a letter holder or other device in upright position. Allow the ImmunoStrip test to remain in the sample extract for 30 minutes. Positive results may be visible in as little as 5 minutes. Lower titer samples may take up to 30 minutes.

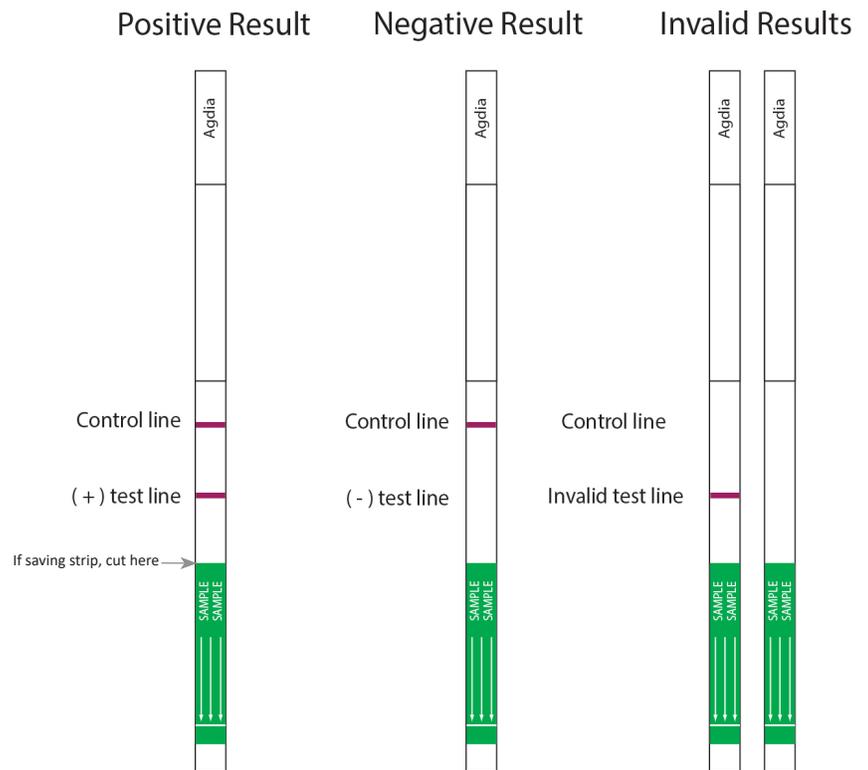
## 7 Interpret Results

Remove test strip from extract and interpret results. Use the images provided as a guide to determine results. If storing the strip as a permanent record, immediately cut the sample pad off the strip, then press the ImmunoStrip between paper towels to remove excess liquid.

If the control line is visible and the test line is also present at any intensity of pink/purple, this indicates a positive\*\* result.

If only the control line is visible, this indicates a negative result. Samples with low levels of bacteria may not be detected with the ImmunoStrip.

The control line assures that the test is working properly. If the control line does not appear, the test is invalid, even if a test line is visible (see troubleshooting).



## SAFETY

ImmunoStrips are non-hazardous. Please refer to SDS for hazards associated with BEB1 buffer: <http://docs.agdia.com/DataSheets.aspx>

## TROUBLESHOOTING

Control line did not develop.	<p>This situation is generally caused by over-submergence of the test strip in the sample extract.</p> <p>Also, ImmunoStrips inserted immediately after extraction and prior to settling for three minutes have an increased chance of device failure due to the possibility of liquid wicking in above the sample line.</p> <p>If no control line is present, results should be considered invalid, and the test should be repeated.</p>
Test runs very slow or not at all.	<p>This can be caused by using too much tissue for extraction. Repeat the test using less tissue or by further diluting your previous sample extract 1:10 with BEB1.</p> <p>If the above is not the case, make sure the test components were warmed to room temperature before use and are within their expiration date.</p>
Test has a green or pigmented test line.	<p>This can be caused by using too much tissue for extraction. Repeat the test using less tissue or by further diluting your previous sample extract 1:10 with BEB1.</p> <p>**In rare cases, the tissue type may cause a pigmented line. Green lines should not be considered a positive result. Red, orange, or purple fruits and tissues (for example, red begonia leaves) may cause what appears to be a positive test line. It is recommended that you contact Agdia before testing these types of samples.</p>
Test and/or control line is weak.	<p>Make sure the test is within its expiration date.</p> <p>If kit contents were left open too long, the strips could have absorbed moisture, which can affect test results. Be sure to always keep the ImmunoStrip vial tightly sealed between uses.</p> <p>The test line may be weak due to low pathogen titer in the sample.</p>