

## USER GUIDE: Agdia ImmunoStrip® and ImmunoComb® Tests

### KIT INFORMATION

#### Intended Use

The ImmunoStrip test is a rapid means of screening crops for the presence of pathogens. ImmunoStrip tests require no equipment or expertise to run. Results are obtained in as little as a few minutes making them perfect for use in the field or greenhouse. The ImmunoStrip must be used with **SEB1** sample extraction buffer.

#### 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.

#### Limitations

There is a potential for false positives when testing ivy geranium samples with CMV, TMV, TSWV, and *Xhp* ImmunoStrips.

#### ImmunoStrip and ImmunoComb Kits (ISK) Include

- ImmunoStrips or ImmunoCombs
- SEB1 sample extraction bags
- User guide

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

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

- Scissors, knife or razor blade
- SEB1 sample extraction buffer
- Sample extraction equipment (e.g., Agdia sample extraction bags; Agdia tissue homogenizer - ACC 00900 or blunt object such as a pen or marker)
- Letter holder or other device to hold sample extraction bags

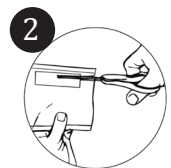
### PERFORMING THE ASSAY (\*Special Attention Required)

#### Prepare Sample



Samples should be taken from symptomatic plant tissue when possible. 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.

**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.**



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

**\*SEB1 Buffer is required to perform this assay.**



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



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.

#### Perform Assay

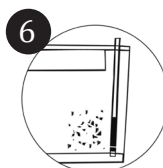


Remove an ImmunoStrip or ImmunoComb then reclose the container. When handling the strips, always grasp the top of the strip marked with the test name. Do not remove the protective covering.

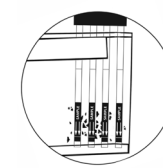


Insert sample end of ImmunoStrip or ImmunoComb into the bag until submerged in the extract up to the white line(s). For best results, insert the ImmunoStrip into the channel portion of the bag (no mesh), or insert the ImmunoComb in front of the mesh layer of the bag. Do not allow the side of the ImmunoStrip 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 strips(s) no more than ¼ inch or to the white line(s) on the ImmunoStrip label.**



Place the bag in a letter holder or other device in upright position. Allow the ImmunoStrip or ImmunoComb 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.



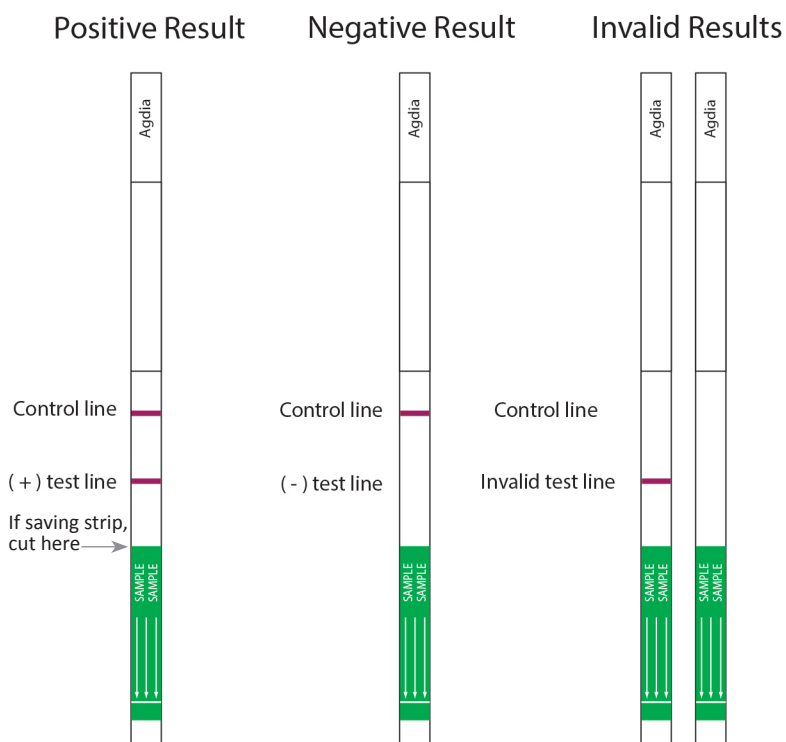
## 7 Interpret Results

Remove test strip(s) from extract and interpret results. Use the images provided as a guide to determine results. Results are interpreted independently for each test in a comb. Please note that individual tests may differ in the amount of time required to see 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.

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 and sample extraction buffer are non-hazardous.

## 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 SEB1.</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 SEB1.</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 or pouch tightly sealed between uses.</p> <p>The test line may be weak due to low pathogen titer in the sample.</p>