ImmunoStrip® User Guide for CymMV & ORSV

On-site Plant Pathogen Testing

Cymbidium mosaic virus and *Odontoglossum ringspot virus* (CymMV & ORSV) ISK/STX 13301

ImmunoStrip®

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Intended Use:

This ImmunoStrip test is a rapid means of screening orchids for all strains of *Cymbidium mosaic virus* and *Odontoglossum ringspot virus* (CymMV & ORSV). The CymMV & ORSV ImmunoStrip is recommended to be used with flower, flower spike, leaf, and root tissue. ImmunoStrip tests require no equipment or expertise to run. Results can be obtained in 30 minutes or less, making ImmunoStrips perfect for use in the field or greenhouse.

Kit Storage:

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

Before use, allow all kit components to warm to room temperature (18 - 30 °C).

Limitations:

Pseudobulb tissue is not recommended to be sampled.

Contents of Kit:

- ImmunoStrips
- *SEB1 sample extraction bags

Not Included but Required:

- Scissors, knife, or razorblade
- Extraction tool
  - » Agdia tissue homogenizer (ACC 00900), marker, or pen
- Letter holder or another device to hold sample extraction bags upright

*Not included if ordering STX only

PERFORMING THE ASSAY (*Special Attention Required)

Prepare Sample

1. Take a sample from symptomatic plant tissue when possible. Agdia sample extract bags **contain 3 mL** of extraction buffer, requiring 0.15 g (approximately ¾ inch^2 or the size of the bottom of the ImmunoStrip container) of flower, leaf, or root tissue for the optimal 1:20 dilution. Flower spike will need 0.03 g for the optimal 1:100 dilution. Since orchid leaves vary in thickness, it is recommended to use a scale to weigh the tissue. Please note that thick or dense tissues can alter the targeted 1:20 dilution. (Figure 1)

Tissue Type	Sample Dilution (weight in g: buffer volume in mL)
Flower	1:20
Flower spike	1:100
Leaf	1:20
Root	1:20

Figure 1

Note: If reusing cutting tools, disinfect them with a 10 % bleach solution between every sample.

2. Cut open the sample extraction bag near the bottom of the label. Be careful not to spill the buffer. ***SEB1** buffer is required to perform this assay. (Figure 2)

Figure 2

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

4. Extract the sample by thoroughly macerating it with an Agdia tissue homogenizer or a blunt object such as a pen or marker. (Figure 3)

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.**

Note: Depending on the sample type, the extract may be too thick to wick up the strip properly. Further dilution may be required. Contact Agdia for sampling assistance.

Figure 3

Perform Assay

5. Remove an ImmunoStrip then reclose the container. When handling the ImmunoStrips, always grasp the top of the ImmunoStrip marked with Agdia's name. Do not remove the protective covering.

Insert sample end of the ImmunoStrip into the channel portion of the bag (no mesh) until submerged in the extract **up to the white line approximately ¼ inch from the bottom**. **Do not allow the side of the ImmunoStrip to come into contact with foam or bubbles (if present)**. (Figure 4)

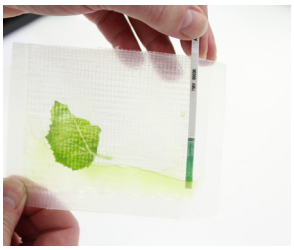


Figure 4

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

Interpret Results

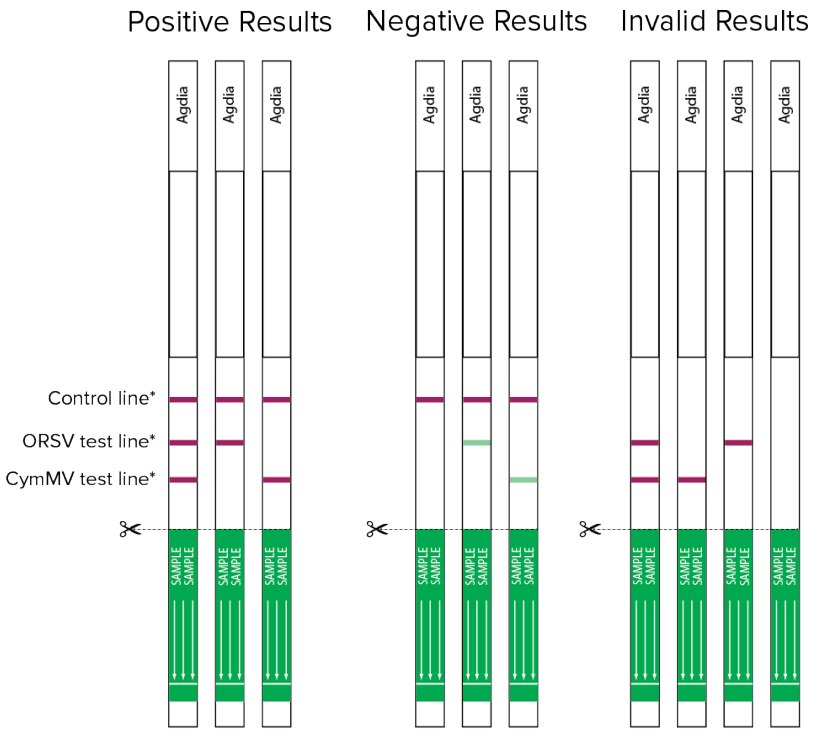
7. Remove the ImmunoStrip from the extract and interpret the results. Use the images provided as a guide to determine results. If storing the ImmunoStrips as a permanent record, immediately cut off the sample pad, then press the remaining ImmunoStrip between paper towels to remove any excess liquid.

If only the control line is visible, this indicates a negative result.

If the control line is visible and the test line is also present at any intensity of pink**/purple, this indicates the presence of the target pathogen (or in some cases, a closely related pathogen). Visit the product webpage to see if any other pathogens are known to cross-react with this test.

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).

As with all diagnostic tools, Agdia recommends confirming all results with a secondary detection method before making any economic decisions (ex: discarding plants due to positive test results, etc.).



*Intensity of valid control and test lines can vary from light pink to dark red/purple.

SAFETY

Agdia recommends reading all relevant SDS sheets before using assay components: http://docs.agdia.com/datasheets.aspx.

TROUBLESHOOTING

Control line did not develop.	• Submerging the ImmunoStrip past the white line in the sample extract. (Step 5) • ImmunoStrip inserted before the 3 minute sample extract settling period. (Step 4)
Test runs very slow or not at all.	• Extracting more tissue than is required. (Step 1) Further dilute sample extract 1:10 in SEB1 and repeat test. • Components were not warmed to room temperature before use. (Kit Storage) • Check kit and components expiration dates.
Test has a green or pigmented test line.	• Extracting more tissue than is required. (Step 1) • Green lines should be considered a negative result. (Step 7) • **In rare cases, red, orange, or purple tissues (for example, red flowers) may cause what appears to be a positive test line. Contact Agdia before testing these types of samples. (Step 7)
Test and/or control line is weak.	• Components absorbed moisture. (Kit Storage) Moisture can cause the membrane to wick without test components and fail to produce lines. • Low pathogen titer in the sample. (Step 7) • Check kit and components expiration dates.

QUESTIONS OR TECHNICAL SUPPORT:

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