

USER GUIDE: CymMV & ORSV ImmunoStrip®
(Product Numbers: ISK 13301, STX 13301)

KIT INFORMATION

Intended Use

The CymMV & ORSV ImmunoStrip® test is a rapid means of screening orchids for all strains of *Cymbidium mosaic virus* and *Odontoglossum ringspot virus*. The CymMV & ORSV ImmunoStrip® must be used with **SEB1** sample extraction buffer for leaf, flower, root and flower spike tissue. Pseudobulb is the only part of the plant not suitable for sampling. Use both symptomatic, as well as non-symptomatic tissue.

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.

ImmunoStrip Kit (ISK) Includes

- ImmunoStrips
- SEB1 sample extraction bags
- User guide

ImmunoStrips (STX 13301/0025) 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



Collect a sample section that is approximately ***3/4 inch square**, about the size of a U.S. Quarter Dollar. Since orchid leaves vary in thickness, it is recommended to use a scale to weigh the tissue. Each mesh bag contains 3 mL SEB1 buffer, which would require 0.15 g leaf, floral or root tissue; or 0.03 g of flower spike tissue. 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.

TISSUE TYPE	SAMPLE DILUTION (tissue weight in g: buffer volume in mL)
Leaf	1:20
Flower (Bud)	1:20
Root	1:20
Flower Spike	1:100



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.

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.

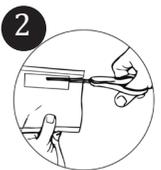
Perform Assay



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 ImmunoStrip into the channel portion (no mesh) of the buffer filled bag until submerged in the extract up to the white line. 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.**

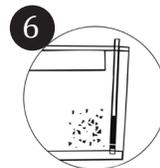


Cut open the sample extraction bag along the top of the label. Be careful not to spill the buffer.

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



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



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.

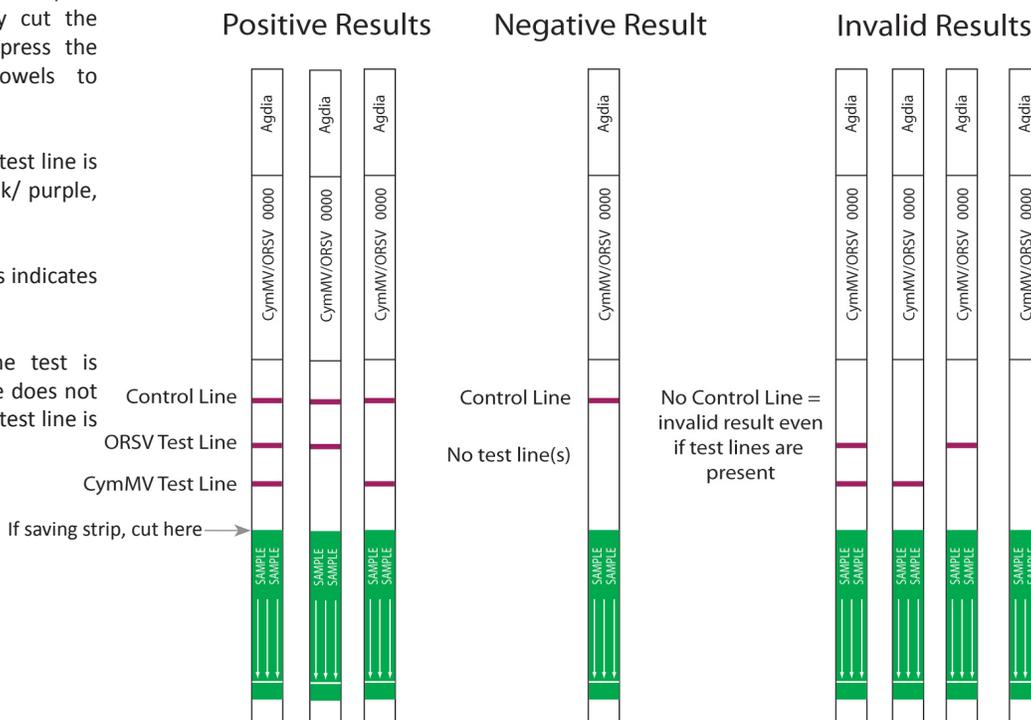
7 Interpret Results

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.

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