ImmunoStrip® Validation Report

On-site Plant Pathogen Testing

Zucchini yellow mosaic virus (ZYMV)

ISK/STX 77700

Phone: 800-622-4342
Sales Email: info@agdia.com
Technical Email: techsupport@agdia.com

Test Characteristics

Test Name	Zucchini yellow mosaic virus
Catalog Number	77700
Acronym	ZYMV
Genus	Potyvirus
Capture Antibody	Polyclonal (Rabbit)
Detection Antibody	Polyclonal (Rabbit)
Format	Lateral Flow Device
Diluents	SEB1
Sample Dilution	1:20

Summary

The Zucchini yellow mosaic virus (ZYMV) ImmunoStrip is used to detect the presence of ZYMV in cucurbit leaves. ZYMV is a member of the Potyvirus genus known for their non-enveloped, flexuous, filamentous virus particles. ImmunoStrips are the perfect screening tool for use in the field, greenhouse, and the lab.

Diagnostic Sensitivity

True Positives	152
Correct Diagnoses	152
Percent	100%

Analytical Sensitivity

Limit of Detection: 1:583,200 dilution of infected tissue (pathogen titer unknown)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

ZYMV-1318 (France)	ZYMV-CA (CA, USA)
ZYMV-CG-04-65 (France)	ZYMV-CH-98-60 (China)
ZYMV-CH-98-77 (China)	ZYMV-CT (CT, USA)
ZYMV-E9 (France)	ZYMV-FL (FL, USA)
ZYMV-HAT (France)	ZYMV-IT (Italy)
ZYMV-MAU (Mauritius)	ZYMV-MAY (Mayotte)
ZYMV-MT7 (Martinique Island)	ZYMV-NAT (Israel)
ZYMV-NY	ZYMV-R5A (Reunion Island)
ZYMV-SIN (Singapore)	ZYMV-SJBCA
ZYMV-Su19 (Sudan)	ZYMV-USDA
ZYMV-WK (France)	ZYMV-Z18

Exclusivity:

Cross-reacts With:

Watermelon mosaic virus (WMV)	

Does Not Cross-react With:

Cucumber vein yellowing virus (CVYV)	Moroccan watermelon mosaic virus (MWMV)
Papaya ring spot virus (PRSV)	Zucchini yellow fleck virus (ZYFV)

Diagnostic Specificity

True Negatives	86
Correct Diagnoses	86
Percent	100%

Selectivity:

No Matrix Effect Observed With:			
Cucumber leaves	Melon leaves	Pumpkin leaves	Squash leaves
Watermelon leaves	Zucchini leaves		

Repeatability

Number of Samples	52
Replicates per Sample	3
Average Percent Agreement Between Replicates	100%

Reproducibility

Number of Samples	40
Replicates per Sample	3
Number of Operators	4
Average Percent Agreement Between Replicates Between Operators	100%

ImmunoStrip® User Guide for ZYMV

On-site Plant Pathogen Testing
***Zucchini yellow mosaic virus* (ZYMV)**
ISK/STX 77700

ImmunoStrip®

Phone: 800-622-4342
Sales Email: info@agdia.com
Technical Email: techsupport@agdia.com

Intended Use:

This ImmunoStrip test is a rapid means of screening cucurbit crops for *Zucchini yellow mosaic virus*. 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.

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:

ZYMV may be present in low concentrations or may be unevenly distributed in the plant. It is important to take samples from tissue showing symptoms to improve your ability to detect the virus. It is best to test new growth on plants showing signs of infection.

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. Symptoms can include leaf deformation, yellowing, shoestring leaves, mottling, mosaic, necrosis, vein-clearing, or distorted leaves. Agdia sample extract bags **contain 3 mL** of extraction buffer, requiring 0.15 g (approximately 1 inch^2 or the size of the bottom of the ImmunoStrip container) of tissue for the optimal 1:20 dilution. Please note that thick or dense tissues can alter the targeted 1:20 dilution. (Figure 1)


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)


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.**

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)

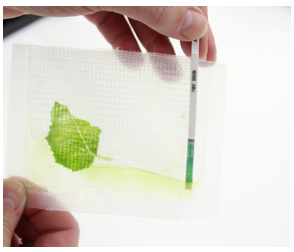


Figure 4

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

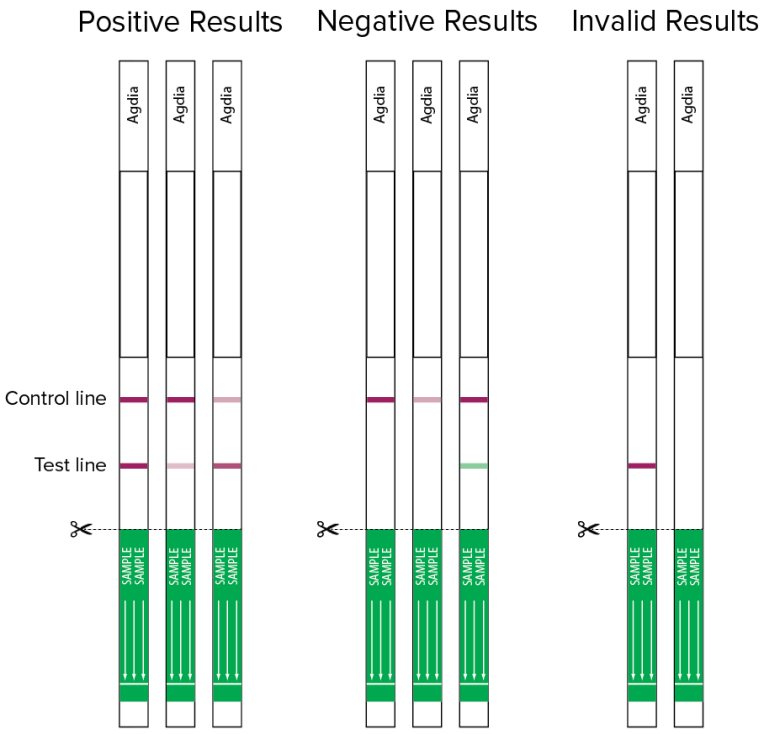
7. Remove the ImmunoStrip from the extract and interpret the results. Use the images provided as a guide to determine results. If storing the ImmunoStrips as a permanent record, immediately cut off the sample pad, then press the remaining ImmunoStrip between paper towels to remove any excess liquid.

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.).



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 fruits and tissues (for example, red cucumbers) 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:

India Contact:

Life Technologies (India) Pvt. Ltd.

Ph: +91-11-42208000, Mobile: +91-9810521400

Email: customerservice@lifetechindia.com Website: www.lifetechindia.com

Scan for How To Video