ImmunoStrips® Validation Report

On-site Plant Pathogen Testing

Cucumber green mottle mosaic virus (CGMMV)

ISK/STX 45702

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Test Characteristics

Test Name	Cucumber green mottle mosaic virus	**Capture Antibody**	Monoclonal (Mouse)
Catalog Number	45702	**Detection Antibody**	Monoclonal (Mouse) and Polyclonal (Rabbit)
Acronym	CGMMV	**Format**	Lateral Flow Device
Genus	Tobamovirus	**Diluents**	SEB1
		Sample Dilution	1:20

Summary

The Cucumber green mottle mosaic virus (CGMMV) ImmunoStrip® is used to detect the presence of CGMMV in cucurbit crops. CGMMV is a member of the Tobamovirus genus known for their rod-shaped virus particles. The ImmunoStrip® is specific to CGMMV and does not cross-react with other viruses that may infect cucurbits. ImmunoStrips® are the perfect screening tool for use in the field, greenhouse, and the lab. The CGMMV ImmunoStrip was redeveloped in 2016 and now offers superior sensitivity compared to other commercially available immunoassay formats, including ELISA.

Diagnostic Sensitivity

True Positives	51
Correct Diagnoses	51
Percent	100%

Analytical Sensitivity

Limit of Detection: 1:10,000,000 dilution of infected tissue (pathogen titer unknown)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

CGMMV ATCC® PV-391™ (Japan)	CGMMV Bulgaria isolate
CGMMV CA, USA isolate	CGMMV Canada isolate
CGMMV France isolate	CGMMV Greece isolate
CGMMV Israel isolate	CGMMV Kuwait isolate
CGMMV Latvia isolate	CGMMV Netherlands isolate
CGMMV Russia isolate	

Exclusivity:

Cross-reacts With:

None known	

Does Not Cross-react With:

Cucumber mosaic virus (CMV)	Groundnut ringspot virus (GRSV)
Impatiens necrotic spot virus (INSV)	Kyuri green mottle mosaic virus (KGMMV)
Melon necrotic spot virus (MNSV)	Melon severe mosaic virus (MeSMV)
Odontoglossum ringspot virus (ORSV)	Papaya ringspot virus (PRSV)
Pepper mild mottle virus (PMMoV)	Piper chlorosis virus (PChV)[1,2]
Ribgrass mosaic virus (RMV)	Squash mosaic virus (SqMV)

Does Not Cross-react With:

Sunn-hemp mosaic virus (SHMV)	Tobacco mosaic virus (TMV)
Tomato brown rugose fruit virus (ToBRFV)	Tomato chlorotic spot virus (TCSV)
Tomato mosaic virus (ToMV)	Tomato ringspot virus (ToRSV)
Tomato spotted wilt virus (TSWV)	Watermelon mosaic virus (WMV)
Zucchini green mottle mosaic virus (ZGMMV)	Zucchini yellow mosaic virus (ZYMV)
[1]Confirmed experimentally at Agdia, Inc.	
[2]Reported to not detect Piper chlorosis virus (PChV), a possible novel Tobamovirus.	

Diagnostic Specificity

True Negatives 63
Correct Diagnoses 63
Percent 100%

Selectivity:

No Matrix Effect Observed With:			
Calibrachoa leaves	Cucumber leaves	Gaura leaves	Heliotrope leaves
Lavender leaves	Melon leaves	Petunia leaves	Portulaca leaves
Pumpkin leaves	Salvia leaves	Scaevola leaves	Watermelon leaves
Zucchini leaves			

Repeatability

Number of Samples 81
Replicates per Sample 2-6
Average Percent Agreement Between Replicates 100%

ImmunoStrip® User Guide
On-site Plant Pathogen Testing
General User Guide
SEB1 Extraction Buffer

ImmunoStrip®

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Intended Use:
ImmunoStrip tests are a rapid means of screening crops for the presence of pathogens. 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:
Extraction and testing of decomposed, dried, or large amounts of tissue can cause invalid results.

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 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)

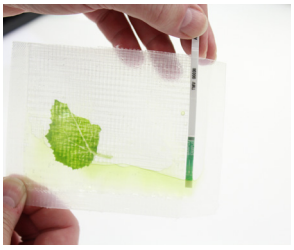


Figure 4

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

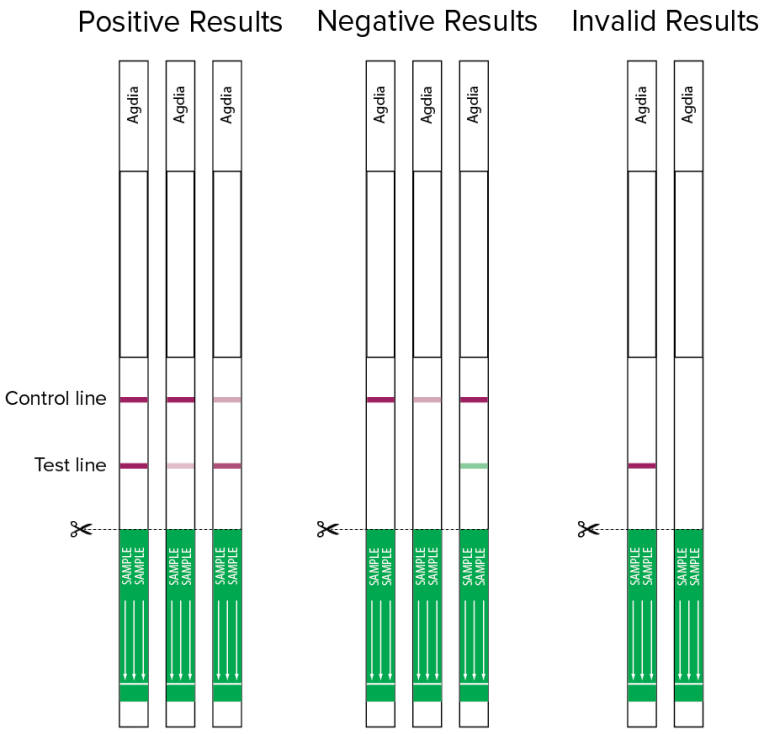
7. Remove the ImmunoStrip from the extract and interpret the results. Use the images provided as a guide to determine results. If storing the ImmunoStrips as a permanent record, immediately cut off the sample pad, then press the remaining ImmunoStrip between paper towels to remove any excess liquid.

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 begonia leaves) 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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