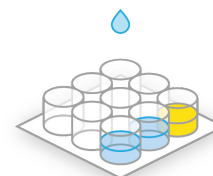


Validation Report: ELISA

PSA/SRA 45702 • *Cucumber green mottle mosaic virus* (CGMMV)



Test Characteristics

Test Name	Cucumber green mottle mosaic virus	Capture Antibody	Polyclonal (Rabbit)
Catalog Number	45702	Detection Antibody	Monoclonal (Mouse)
Acronym	CGMMV	Format	Compound-ELISA
Genus	Tobamovirus	Diluents	GEB/ECI
		Sample Dilution	1:10

Summary

This ELISA test is a qualitative serological assay for the detection of Cucumber green mottle mosaic virus (CGMMV), the causal agent of Cucumber green mottle mosaic disease, in cucurbit crops including cucumber, melon, and watermelon leaves and seeds. CGMMV is a member of the Tobamovirus genus known for their rod-shaped virus particles.

Diagnostic Sensitivity

True Positives	51
Correct Diagnoses	51
Percent	100.0%

Analytical Sensitivity

Limit of Detection: 1:194,400 dilution of infected tissue (pathogen titer unknown)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

CGMMV ATCC® PV-391™ (Japan)	CGMMV-CG001 (CA, USA)
CGMMV-CG002 (CA, USA)	CGMMV-CG003 (CA, USA)
CGMMV-CG004 (CA, USA)	CGMMV-CG006 (Israel)
CGMMV-CG007 (Netherlands)	CGMMV-CG008 (Netherlands)
CGMMV-CG009 (Netherlands)	CGMMV-CG010 (Netherlands)
CGMMV-CG011 (Netherlands)	CGMMV-CG012 (Netherlands)
CGMMV-CG013 (Netherlands)	CGMMV-CG014 (Netherlands)
CGMMV-CG015 (Netherlands)	CGMMV-CG019 (Thailand)
CGMMV-CG020 (Netherlands)	CGMMV-CG021 (Netherlands)
CGMMV-CG022 (Netherlands)	CGMMV-CG023 (Netherlands)
CGMMV-CG025 (Latvia)	CGMMV-CG026 (France)
CGMMV-CG027 (France)	CGMMV-CG028 (Bulgaria)
CGMMV-CG029 (Greece)	CGMMV-CG030 (Greece)
CGMMV-CG031 (Netherlands)	CGMMV-CG032 (Russia)
CGMMV-CG033 (Netherlands)	CGMMV-CG034 (Netherlands)
CGMMV-CG035 (Kuwait)	CGMMV-CG036 (Canada)
CGMMV-CG038 (China)	



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)
Sunn-hemp mosaic virus (SHMV)	Tobacco mosaic virus (TMV)
Tomato chlorotic spot virus (TCSV)	Tomato mosaic virus (ToMV)
Tomato ringspot virus (ToRSV)	Tomato spotted wilt virus (TSWV)
Watermelon green mottle mosaic virus (WGMMV) ³	Zucchini green mottle mosaic virus (ZGMMV) ³
Zucchini yellow mosaic virus (ZYMV)	

¹Confirmed experimentally at Agdia, Inc.²Reported to not detect Piper chlorosis virus (PChV), a possible novel Tobamovirus.³Reported to not cross-react**Diagnostic Specificity**

True Negatives 40
 Correct Diagnoses 40
 Percent 100.0%

Selectivity:**No Matrix Effect Observed With:**

Bean leaves	Hosta leaves	Pumpkin leaves	Watermelon leaves
Begonia leaves	Impatiens leaves	Pumpkin seeds	Watermelon seeds
Blueberry leaves	Melon leaves	Soybean leaves	Zinnia leaves
Cucumber leaves	Melon seeds	Squash leaves	
Cucumber seeds	Pepper leaves	Squash seeds	
Dianthus leaves	Petunia leaves	Tomato leaves	



User Guide: Compound-ELISA PathoScreen® Kit

General User Guide • GEB / ECI • Alkaline Phosphatase

Test Principle, Intended Use and Limitations

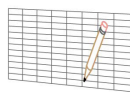
This product is intended for the qualitative detection of the target analyte via a direct, triple antibody sandwich protocol known as Compound-ELISA. Upon successful completion of the test, samples containing the target analyte will turn yellow, due to the alkaline phosphatase enzyme label, while negatives will remain colorless. Visit the product webpage for information regarding host reactions, cross-reactions, or other limitations.

Handling Information

Antibodies and plates should be stored refrigerated (2 - 8 °C) between uses. All test materials should be warmed to room temperature (18 - 30 °C) before use. For materials provided please see the product webpage. Do not store 1X buffers for more than one day.

Safety

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



Test Preparation

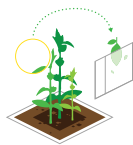
1. Visit the product webpage to view [buffer instructions](#), [logsheet](#), and other documents.
2. Record lot numbers of materials to be used in the test using the logsheet.
3. Prepare a humid box by lining an airtight container with a wet paper towel.
4. Mix both concentrated and diluted antibodies thoroughly before each use.

Scan for
buffer
instructions



Positive and Negative Control Preparation

1. Use General Extract Buffer (GEB) to hydrate fresh controls, according to label, at least five minutes before use.
2. Recap and mix thoroughly.
3. Use of frozen or aliquoted controls comes with increased stability risks and may not match expected O.D. values.



Sample Preparation and Plate Loading

1. Sample symptomatic tissue if possible. Other plant parts may be tested, including asymptomatic tissue.
2. At the time of testing, grind and dilute the samples at a 1:10 ratio with GEB.

Example: 0.3 g plant tissue, extracted with 3 mL of GEB.

3. Dispense 100 µL of the extracted samples, positive control, negative control, and extraction buffer into the provided antibody coated microtiter plate following your logsheet.
4. Incubate plate in the humid box for either 2 hours at room temperature or overnight at 2 - 8 °C.



Prepare Detection Solution

1. Prepare the mix of the detection antibody (Bottle A) and enzyme conjugate (Bottle B) in a non-binding container, such as Agdia's sample cups (ACC 00960).
2. Dilute both the thoroughly-mixed Bottle A and Bottle B, per the dilution on the labels, in 1X ECI buffer (see example). You will need 100 µL of diluted detection solution per well; a full plate will need 10 mL.

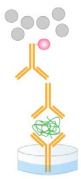
Example: (Wells Used 16 x 100 µL) ÷ 100[†] = 16[‡] µL Bottle A and Bottle B

[†]Bottle dilution will be either 100 or 200

[‡]Add 16 µL of both Bottle A and Bottle B into 1X ECI

3. Wash the sample from the plate 8 times using 1X PBST.
4. Tap plate dry using lint-free paper towel.
5. Thoroughly mix and pipette 100 µL of the diluted detection solution into each testwell.
6. Incubate plate in the humid box for 2 hours at room temperature.



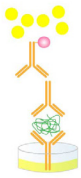


Prepare Substrate

1. Add 1 PNP substrate tablet per 5 mL of 1X PNP substrate buffer into a dedicated container and keep in the dark until use. You will need 100 μ L of diluted PNP solution per well; a full plate will need 10 mL. Ensure tablets are dissolved before use.
2. Wash the detection solution from the plate 8 times using 1X PBST.
3. Tap plate dry using lint-free paper towel.
4. Pipette 100 μ L of dissolved PNP solution into each testwell.
5. Incubate, protected from light, for 1 hour at room temperature.

Interpreting Results

1. Visually inspect wells and remove bubbles, if present. Measure O.D. values with a spectrophotometer at 405 nm or 405 nm with a 650 nm blank.
2. The test is valid if the positive and negative control O.D. results meet expected values (see Certificate of Analysis).
3. Sample interpretations should be performed on a case-by-case basis. Plant tissue interactions with ELISAs can vary greatly between plant species and even varieties. Certain healthy tissues can cause an elevated or higher than normal O.D. value. In this case, a healthy sample(s) of the same species or variety is needed to determine the healthy average.
4. Generally, positive and negative thresholds can be determined by using 2 times the healthy average. Any samples with an O.D. value higher than 2 times the healthy average are positive, and samples with an O.D. value below 2 times the healthy average are negative. An alternative method for threshold calculations is the healthy average plus 3 times the standard deviation of the healthy sample set.



Method 1	Healthy Avg.	0.105	2 x Healthy Avg.	0.210
	Sample 1	0.355 (Positive)	Sample 2	0.190 (Negative)

Method 2	Healthy Avg.	0.105	Std. Dev.	0.030	Healthy Avg. + 3 x Std. Dev.	0.195
	Sample 1	0.355 (Positive)	Sample 2	0.190 (Negative)		

5. Positive O.D. values indicate 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. 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.).

Warranty

Agdia reagents are warranted for performance issues that arise from manufacturer defect. See product packaging for relevant expiration dates. Agdia's return policy can be found at www.agdia.com/customer-support/return-policy.

Additional Information

If you would like more information on how to run ELISA, please see Agdia's FAQ section, <http://www.agdia.com/customer-support/frequent-questions-and-troubleshooting>. For further documentation, including this user guide, buffer formulations, and a logsheet, please see Agdia's specific product webpages. For answers to your technical questions, please contact us at techsupport@agdia.com.

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