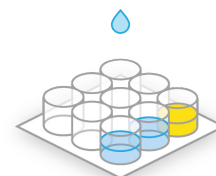


Validation Report: ELISA

BRA 44001 • *Clavibacter michiganensis* subsp. *michiganensis* (Cmm)



Test Characteristics

Test Name	Clavibacter michiganensis subsp. michiganensis	Capture Antibody	Polystyrene Plate
Catalog Number	44001	Detection Antibody	Monoclonal (Mouse)
Acronym	Cmm	Format	ACP-ELISA
Genus	Clavibacter	Diluents	CCB/MPBST
		Sample Dilution	Bacterial Streaming

Summary

This ELISA test is a qualitative serological assay for the detection of *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), the causal agent of bacterial canker in tomato. This ELISA can be used to test bacterial cultures and tissue soaks. Cmm is a member of the *Clavibacter* genus known for their aerobic, non-motile, Gram-positive, non-spore, curved rod shaped bacteria.

Diagnostic Sensitivity

True Positives	12
Correct Diagnoses	12
Percent	100%

Analytical Sensitivity

Limit of Detection: 7.9×10^4 CFU/mL

Analytical Specificity

Inclusivity:

This assay was designed to detect all strains and isolates of Cmm. Twelve distinct samples of Cmm have been experimentally proven to be detected.

Exclusivity:

Cross-reacts With:

Clavibacter michiganensis subsp. nebraskensis	Clavibacter michiganensis subsp. sepedonicus (mucoid)
Xanthomonas campestris pv. campestris	

Does Not Cross-react With:

Acidovorax avenae pv. avenae	Pseudomonas syringae pv. tomato
Acidovorax avenae pv. citrulli	Spiroplasma citri
Agrobacterium radiobacter	Stenotrophomonas maltophilia
Agrobacterium rhizogenes	Xanthomonas albilineans
Agrobacterium vitas	Xanthomonas arboricola pv. celebensis
Agrobacterium tumefaciens	Xanthomonas arboricola pv. pruni
Burkholderia glumae	Xanthomonas axonopodis pv. begoniae
Clavibacter michiganensis subsp. insidiosus	Xanthomonas axonopodis pv. citri
Clavibacter michiganensis subsp. tessellarius	Xanthomonas axonopodis pv. diffebachia



Does Not Cross-react With:

Curtobacterium flaccumfaciens subsp. poinsettiae	Xanthomonas axonopodis pv. phaseoli
Dickeya chrysanthemi	Xanthomonas campestris pv. armoraciae
Erwinia amylovora	Xanthomonas campestris pv. zinnea
Pantoea agglomerans	Xanthomonas citri pv. aurantifolii
Pantoea stewartii	Xanthomonas citromelo
Pectobacterium atrosepticum	Xanthomonas hortorum pv. pelargonii
Pectobacterium carotovora pv. carotovora	Xanthomonas oryzae pv. oryzae
Pseudomonas fuscovaginae	Xanthomonas translucens pv. translucens
Pseudomonas savastanoi pv. glycineae	Xanthomonas vesicatoria
Pseudomonas savastanoi pv. phaseolicola	Xylella fastidiosa
Pseudomonas syringae pv. syringae	

Diagnostic Specificity

True Negatives 45
 Correct Diagnoses 45
 Percent 100%

Selectivity:

No Matrix Effect Observed With:			
Bacterial Cultures	Tissue soaks	Tomato	



User Guide: ACP-ELISA Bacterial Reagent Set

General User Guide • CCB / MPBST • Alkaline Phosphatase

Test Principle, Intended Use and Limitations

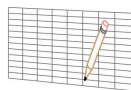
This product is intended for the qualitative detection of the target analyte via an antigen-coated plate protocol known as ACP-ELISA. Upon successful completion of the test, samples containing the target analyte will turn yellow, due to the alkaline phosphatase enzyme label, while negatives will remain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

Handling Information

Antibodies 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. The buffers necessary to run this assay can be purchased as buffer pack ACC 00112. 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 formulations](#), [logsheet](#), and other documents.
2. Record lot numbers of materials to be used in the test using the logsheet.
3. Mix both concentrated and diluted antibodies thoroughly before each use.

Scan for buffer formulations



Positive and Negative Control Preparation

1. Use 1X carbonate coating buffer (CCB) 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.



Plant Sample Preparation

1. Sample symptomatic vascular tissue if possible. Asymptomatic vascular tissue may be tested as well.
2. Cut sample and place in 1 mL of water to produce a bacterial stream. After 30 minutes remove plant vascular tissue.
3. Centrifuge bacterial stream at > 10,000 rpm for at least 2 minutes, remove water, and resuspend pellet in 200 µL 1X CCB.
4. Alternatively, serially dilute the water in ELISA wells with 1X CCB.

Example:

1. Pipette 150 µL of the water sample into well A1.
2. Pipette 100 µL of 1X CCB into A2 through A8.
3. Pipette 50 µL from A1 into A2
4. Repeat; pipette 50 µL from A2 into A3. Repeat until you reach A7.

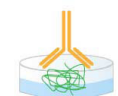


Bacterial Culture Sample Preparation

1. Collect sample from solid media using a sterile toothpick or loop, such as Agdia's 1 µL loops (ACC 00077).
2. Add sample to 1 mL of 1X CCB and agitate.
3. For a more quantitative sampling, measure the sample's optical density (OD) at 600 nm and adjust OD to 0.01.
 - a. An OD of 0.01 at 600 nm is equivalent to about 10⁷ CFU/mL.

Sample Incubation

1. Dispense 100 µL of the extracted samples, positive control, negative control, and 1X CCB into the plate following your logsheet.
2. Incubate plate overnight in an incubator at 37 °C.
3. Be sure testwells are completely dry before continuing.
4. Prepare a humid box by lining an airtight container with a wet paper towel.
5. Prepare 1X MPBS buffer and add 200 µL into each testwell
6. Incubate plate in the humid box for 30 minutes at room temperature.



Prepare Detection Antibody

1. Prepare the detection antibody in a non-binding container, such as Agdia's sample cups (ACC 00960).
2. Dilute the thoroughly-mixed detection antibody, per the dilution on the label, in 1X MPBST buffer (see example). You will need 100 µL of diluted detection antibody per well; a full plate will need 10 mL.

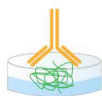
Example: (Wells Used 16 x 100 µL) ÷ 200 = 8 µL Detection Antibody
Bottle dilution will be either 100 or 200



m44.3

Revised: 09/23/2020

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Prepare Detection Antibody (Continued)

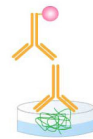
- Wash the MPBS from the plate 3 times using 1X PBST.
- Tap plate dry using lint-free paper towel.
- Thoroughly mix and pipette 100 μ L of the diluted detection antibody into each testwell.
- Incubate plate in the humid box for 1 hour at room temperature.

Prepare Enzyme Conjugate

- Prepare the enzyme conjugate, in a non-binding container, such as Agdia's sample cups (ACC 00960).
- Dilute the thoroughly-mixed enzyme conjugate, per the dilution on the label, in 1X MPBST buffer (see example). You will need 100 μ L of diluted enzyme conjugate per well; a full plate will need 10 mL.

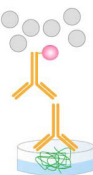
*Example: (Wells Used $16 \times 100 \mu\text{L}$) \div 200 = $8 \mu\text{L}$ Enzyme Conjugate
 'Bottle dilution will be either 100 or 200*

- Wash the detection antibody from the plate 8 times using 1X PBST.
- Tap plate dry using lint-free paper towel.
- Thoroughly mix and pipette 100 μ L of the diluted enzyme conjugate into each testwell.
- Incubate plate in the humid box for 1 hour at room temperature.



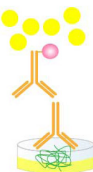
Prepare Substrate

- 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.
- Wash the ECA from the plate 8 times using 1X PBST.
- Tap plate dry using lint-free paper towel.
- Pipette 100 μ L of dissolved PNP solution into each testwell.
- Incubate, protected from light, for 1 hour at room temperature.



Interpreting Results

- 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.
- The test is valid if the positive and negative control O.D. results meet expected values (see Certificate of Analysis).
- 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.
- 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)		

- 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.

