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.

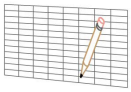
Handling Information

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.

Scan for buffer formulations



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.

Positive and Negative Control Preparation

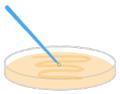
1. Use 1X carbonate coating buffer (CCB) to hydrate fresh controls, according to label, at least five minutes before use.
2. Recap and mix thoroughly.
3. Use of frozen or aliquoted controls comes with increased stability risks and may not match expected O.D. values.

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.

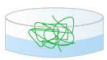
Example:
1. Pipette 150 µL of the water sample into well A1.
2. Pipette 100 µL of 1X CCB into A2 through A8.
3. Pipette 50 µL from A1 into A2
4. Repeat; pipette 50 µL from A2 into A3. Repeat until you reach A7.

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 10^7 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

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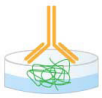
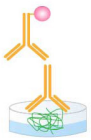


Prepare Detection Antibody (Continued)

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

Prepare Enzyme Conjugate

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

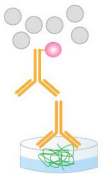


Example: (Wells Used 16 x 100 µL) ÷ 200' = 8 µL Enzyme Conjugate

'Bottle dilution will be either 100 or 200

3. Wash the detection antibody 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 enzyme conjugate into each testwell.
6. Incubate plate in the humid box for 1 hour at room temperature.

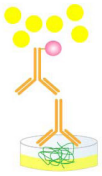
Prepare Substrate



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 µL of diluted PNP solution per well; a full plate will need 10 mL. Ensure tablets are dissolved before use.
2. Wash the ECA from the plate 8 times using 1X PBST.
3. Tap plate dry using lint-free paper towel.
4. Pipette 100 µL 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 warrantied 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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