User Guide: DAS-ELISA Reagent Set

SRP 33900 • *Ralstonia solanacearum* (Rs) • GEB / 1X Enzyme Conjugate • Peroxidase

Test Principle, Intended Use and Limitations

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

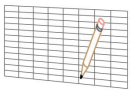
Handling Information

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 00222. 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, 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 formulations

Prepare Capture Antibody

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

   Example: (Wells Used 16 x 100 µL) ÷ 200[†] = 8 µL Capture Antibody

   [†]Bottle dilution will be either 100 or 200

3. Thoroughly mix and pipette 100 µL of diluted CAB into each testwell of the provided high-bind microtiter plate.
4. Incubate plate in the humid box for either 4 hours at room temperature (18 - 30 °C) or overnight at 2 - 8 °C.
5. Coated plates should be used within 24 hours.

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

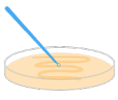
1. Sample symptomatic crown or root tissue if possible. Other plant parts may be tested, including asymptomatic tissue.
2. Cut a 1/4 inch cross section using a clean knife or razor blade.
3. Place the entire cross section in 1mL of GEB.
4. Soak for 10 minutes.
5. Alternatively, grind and dilute the samples at a 1:10 ratio with GEB.

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

Tuber Sample Preparation

1. Extract a core sample using a 1 mL pipette tip to remove a section approximately 0.5 cm deep from the stolon (stem) end of the tuber.
2. Place the core into 3 mL of GEB and completely crush.

Bacterial Culture Sample Preparation



1. Collect a sample using a sterile loop or similar object, such as Agdia's 1 µL loops (ACC 00077).
2. Add the looped sample to 1 mL of GEB 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 10^7 cfu / mL.

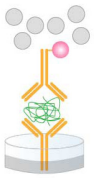
Agdia, Inc.
52642 County Road 1
Elkhart, IN 46514
574-264-2014 / 800-622-4342
www.agdia.com / info@agdia.com

Plate Loading

1. Empty coated plate contents and wash 3 times with 1X PBST.
2. Tap plate dry using lint-free paper towel.
3. Dispense 100 µL of the extracted samples, positive control, negative control, and extraction buffer into the plate following your logsheet.
4. Incubate plate in the humid box for 1 hour at room temperature

Prepare Enzyme Conjugate

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



Prepare Substrate

1. TMB is a ready to use solution. Keep in the dark until use.
2. Wash the ECP from the plate 8 times using 1X PBST.
3. Tap plate dry using lint-free paper towel.
4. Pipette 100 µL of TMB into each testwell.
5. Incubate, protected from light, for 15 minutes at room temperature.

Interpreting Results

1. Visually inspect wells and remove bubbles, if present. Measure O.D. values with a spectrophotometer at 650 nm.
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.*	*0.065*	*2 x Healthy Avg.*	*0.130*
	Sample 1	*0.255 (Positive)*	*Sample 2*	*0.115 (Negative)*

Method 2	*Healthy Avg.*	*0.065*	*Std. Dev.*	*0.030*	*Healthy Avg. + 3 x Std. Dev.*	*0.155*
	Sample 1	*0.255 (Positive)*	*Sample 2*	*0.115 (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.

Agdia, Inc.
52642 County Road 1
Elkhart, IN 46514
574-264-2014 / 800-622-4342
www.agdia.com / info@agdia.com