Ralstonia solanacearum PathoScreen® Kit

DAS ELISA, peroxidase label
Catalog number: PSP 33900

List of contents

Lot number	Item	96 wells	288 wells	480 wells
_____	Antibody–coated 96-well microtiter plates	1	3	5
_____	Peroxidase enzyme conjugate, ready to use	11 ml	33 ml	55 ml
_____	TMB substrate solution	25 ml	40 ml	60 ml
_____	Positive control, ready to use (if available)	1	1	1
	The above items should be stored at 4°C			
_____	PBST wash buffer, 20X concentrate, 50 ml	3	5	7
_____	Tween-20	15 ml	30 ml	30 ml
_____	General extract buffer (GEB)	16.5 g	33 g	33 g
	The above items should be stored at room temperature.			

Materials required, but not provided

- Distilled or purified water
- Paper towels
- Micropipette
- Micropipette tips
- Cork borer
- Lyophilized negative control can be purchased from Agdia
- Sample grinding device such as:
  - Agdia sample mesh bag (ACC 00930)
  - Agdia tissue homogenizer (ACC 00900)
  - Mortar and pestle
- Airtight container for incubations

Limitations

Expiration: This test should be used within 1 year of purchase.

Storage: Test results may be weak or the test may fail if storage instructions are not followed properly.

Buffers: Do not store 1X buffers from day to day. Buffers should be warmed to room temperature prior to use. Buffer formulations on page 5 are for reference only.

Dilutions: Read all labels carefully prior to preparing solutions to assure proper antibody concentrations. All antibody dilutions have been optimized for the greatest possible sensitivity and specificity based on available isolates and hosts. Using dilutions other than those listed can lead to potential false positives or false negatives.

Sensitivity: The *Rs* may be present in low concentrations or may be unevenly distributed in the plant. It is important to take samples from tissue showing symptoms to improve your ability to detect the pathogen. The lower detection limit of this test is about 1.0×10^5 cfu/mL.

Precautions

Prevent direct skin and eye contact with, or ingestion of, product components. Obtain medical attention in case of accidental ingestion of kit components. Always wash hands thoroughly after using this product.

Preparing for the test

Familiarize yourself with the kit components. Check that all components are present in the kit.

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Technical service

If you have any questions about using this kit, please contact Agdia, Inc. Monday – Friday by phone (574-264-2014 or 1-800-622-4342) or by email (info@agdia.com)

Intended Use

The Ralstonia solanacearum (*Rs)* ELISA test is intended for use with plant samples exhibiting symptoms of *Rs*. The test can also be used to test bacterial culture samples. The *Rs* ELISA detects *Rs* to the species level and cannot differentiate race or biovar.

Test Principle

The *Rs* test system uses a direct, double antibody sandwich protocol known as DAS ELISA. Monoclonal antibodies to EPS of *Rs* are coated to the testwells of a microplate. Next, samples are added to the microplate. If EPS is present in the sample, it is bound by the antibodies and captured on the microplate during the incubation period. After incubation, the plate is washed to remove unbound sample. An enzyme conjugate solution, containing a monoclonal antibody conjugated to peroxidase, is added and binds to any captured EPS. After incubation the plate is washed to remove any unbound conjugate. This final binding creates a sandwich of the target analyte between the two specific antibodies. TMB substrate is added to the microplate. If the peroxidase conjugate is present a blue color will be produced indicating the presence of EPS. Buffer wells and negatives should remain colorless. The color reactions can be observed visually or measured with a spectrophotometer.

Prepare buffers

Prepare only as much 1X buffers that will be needed for one day.

PBST wash buffer

Prepare PBST wash buffer by diluting one 20X pouch of PBST wash buffer with 950 ml of distilled water.

General extract buffer (GEB)

GEB general extract buffer is used to dilute and extract samples. It is used at a sample to buffer ratio of 1:10 (tissue weight in g: buffer volume in ml).

Buffer powder	16.5 g
Distilled water	500 ml
Tween-20	10.0 g

Add about 10 ml of water to the powder and mix into smooth slurry. While mixing, slowly add the remaining volume of water. Add Tween-20 to the solution.

This buffer contains 0.02% sodium azide as a preservative.

Optional: Adjust the pH of the 1X buffer until the pH is within the range of 7.2 to 7.8. Store at 4°C when not in use.

Prepare testwells

If you will be using less than a full 96-well plate, remove any unused strips and seal them in the foil pouch with the desiccant. Using a permanent marker, number the strips in case a strip becomes separated from the frame.

Prepare a humid box by lining an airtight container with a wet paper towel. Keeping testwells in a humid box during incubation will help prevent samples from evaporating.

Make a copy of the loading diagram and record the locations of your samples and controls. We recommend that you use a buffer well and positive control well on each plate each time you run the test.

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Preparing samples

Plant samples

It is best to take samples from the crown region (just above the soil line) and roots of the plant. It is in these areas where the pathogen is concentrated. However, all parts of the symptomatic plant can be tested. Symptoms include wilting or abnormal yellowing.

Cut ¼ inch cross sections from crown, stem, petiole (above soil line), or roots using a knife or razor blade. Place the entire cross section piece into a sample extraction bag or tube with 1 ml of GEB sample extraction buffer and allow sample to soak 10 minutes. Wash the knife or razor blade with bleach between each sample.

When testing tubers, use a disposable 1 ml plastic pipette tip to remove a core section approximately 0.5cm deep. Take the sample from the stolon end of the tuber. Insert the sample between the layers of mesh lining of an Agdia sample mesh bag (ACC 00930). Dispense 3 ml of GEB into the bag. Using a blunt object, completely crush the sample. Agdia recommends only one sample per bag to achieve optimal results.

Bacterial culture samples

Lift a colony or part of a colony with a sterile loop or toothpick and stir into a screw cap vial containing 1 ml of GEB sample extraction buffer.

If using cell culture broth, adjust the cells suspension to a concentration of 10^5 to 10^{12} cfu/mL by measuring the solution's optical density at 600 nm and adjusting to 0.01.

Test Procedure

1. Dispense samples

Following your loading diagram, dispense 100 µl of prepared sample into sample wells. Dispense 100 µl of positive control into positive control wells, and dispense 100 µl of GEB sample extraction buffer into buffer wells. If you will be using a negative control, dispense 100 µl into negative wells.

2. Incubate plate

Set the plate inside the humid box and incubate for 1 hour at room temperature.

3. Warm enzyme conjugate

About 15 minutes before the incubation is complete, remove the bottle of peroxidase enzyme conjugate from the refrigerator. Shake bottle to mix contents thoroughly. Measure the volume of peroxidase enzyme conjugate needed for the test. Immediately return unused conjugate to refrigerator. You will need 100 µl of peroxidase enzyme conjugate for each testwell you are using. To estimate the volume needed, measure 1 ml for each 8 well strip used. A full plate will require about 10 ml.

4. Wash plate

When the sample incubation is complete, wash the plate. Use a quick flipping motion to dump the wells into a sink or waste container without mixing the contents.

Fill all the wells completely with 1X PBST, and then quickly empty them again. Repeat 7 times.

After washing, hold the frame upside down and tap firmly on a folded paper towel to remove all droplets of wash buffer.

Inspect the testwells. All wells should be free of plant tissue. If tissue is present repeat the wash step and tap firmly on a paper towel.

5. Add enzyme conjugate	Dispense 100 µl of peroxidase enzyme conjugate per well.
6. Incubate plate	Incubate the plate in the humid box for 1 hour at room temperature.
7. Warm TMB substrate solution	About 15 minutes before the end of the above incubation step, measure the required amount of TMB substrate needed. Return the remaining TMB substrate to the refrigerator. Allow measured TMB substrate to warm to room temperature. Caution: TMB substrate is light sensitive, extra precautions are necessary to protect it from light sources when warming to room temperature. You will need 100 µl of substrate for each testwell you are using. To estimate the volume needed, measure 1 ml for each 8 well strip used. A full plate will require about 10 ml.
8. Wash plate	As before, wash the plate 8 times with 1X PBST. Inspect the wells looking for the presence of air bubbles. Tap firmly on the paper towel to remove remaining wash buffer and any air bubbles. If air bubbles are still present they may be broken with a clean pipette tip.
9. Add TMB substrate solution	Dispense 100 µl of TMB substrate into each testwell.
10. Incubate plate	Incubate the plate in a humid box for 15 minutes.
11. Evaluate results	Examine the wells by eye, or measure on a plate reader at 650 nm. Air bubbles which are present at the time of reading can alter results, if in the light path. Agdia recommends that bubbles be eliminated prior to reading. Wells in which a blue color develops indicate positive results. Wells in which there is no significant color development indicate negative result. Test results are valid only if positive control wells give a positive result and buffer wells remain colorless. Results may be interpreted after more than 15 minutes of incubation as long as negative wells remain virtually clear.

Buffer Formulations

PBST Buffer (Wash Buffer) (1X)

Dissolve in distilled water to 1000 ml:

Sodium chloride	8.0 g
Sodium phosphate, dibasic (anhydrous)	1.15 g
Potassium phosphate, monobasic (anhydrous)	0.2 g
Potassium chloride	0.2 g
Tween-20	0.5 g

Adjust pH to 7.4

General Extract Buffer (GEB 1X)

Dissolve in 1000 ml of 1X PBST:

Sodium sulfite (anhydrous)	1.3 g
Polyvinylpyrrolidone (PVP) MW 24-40,000	20.0 g
Sodium azide	0.2 g
Powdered egg (chicken) albumin, Grade II	2.0 g
Tween-20	20.0 g

Adjust pH to 7.4. Store at 4°C.

Date _____ Test _____

Test performed by _____

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B												
C												
D												
E												
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