adgen phytodiagnostics

AGRISCREEN® – *Phytophthora* spp

96 WELL ELISA DETECTION KIT

Introduction – Key Information

For laboratory use only. Not for drug, household or other use.

Information generated from the use of this kit may be used as a plant disease aid. This kit is not intended for use as an exclusive diagnostic procedure.

The entire protocol should be carefully reviewed before starting the assay.

Intended Use

This kit is intended to screen plant tissue and/or cultured materials for the detection of *Phytophthora* spp. This kit is for research use only. Information generated may be used as a detection aid but should not be relied upon exclusively when disease diagnosis is being made.

Principle of the Assay

This kit is based upon a double antibody sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) The microwells of the ELISA plate have been pre-coated with a purified preparation of specific antibodies. The plant tissue is extracted and added to the antibody-coated wells. As levels of the target pathogen in the sample increase, the amount of pathogen that is bound by the antibody attached to the well increase. The pathogen remaining bound to the antibody-coated well is determined by adding a fixed amount of enzyme-conjugate (the enzyme in this case is horseradish peroxidase). The bound enzyme activity is measured by the addition of the substrate, which develops a blue colour in the presence of the enzyme. The colour development is directly proportional to the amount of pathogen in the original extract.

Materials Provided

Product Description	Quantity Supplier
Sensitised strips	- 12 strips
Non-sensitised micro well strips	- 4 strips
Filter Extraction Bags	- 50 bags
5xs Extraction solution	- 40mls
20xs Wash Solution	- 50mls
Positive Control	- 2mls
Negative Control	- 2mls
HRP Conjugate	- 14mls
Substrate	- 14mls
Stop Solution	- 14mls

Materials Required but not Provided

Micropipette (50-100µl)
Micro well plate shaker
Micro well plate reader (650nm)

Precautions

- Allow kit to warm to room temperature before use. Store kit at 2-8^{o}C when not in use. Do not freeze.
- All contents should be mixed thoroughly by repeated gentle inversion (do not shake).
- Avoid exposing test kit to heat for extended periods.
- Do not use test kit beyond expiration date.
- Ensure that un-used strips are placed back in the foil pouches with the desiccant bag and the end of the pouch folder over and sealed with tape.
- Do not mix reagents from one batch with another.

Kit Preparation

5xs extraction solution:

- The extraction solution is supplied as a 5xs concentrate therefore for every 5ml of working strength extraction solutions required 1ml should be taken and 4mls of distilled or deionised water added in order to give a working strength solution.
- For each strip 4 samples can be tested in duplicate therefore 16mls of working strength buffer will be required, i.e. 3.25mls concentrate plus 13mls of water.
- The pH of the working strength extraction solution should be in the range pH7.1 - 7.3.

20xs wash solution:

- This buffer is supplied as a 20xs concentrate therefore for every 1ml, 19mls of distilled or deionised water should be added in order to give a working strength solution.
- For each strip 50mls of working strength buffer will be required, i.e. 2.5mls concentrate plus 47.5mls of water.
- The pH of the working strength wash solution should be in the range pH7.7 – 7.9.

Substrate:

- The substrate is supplied ready to use.
  Note: Care should be taken not to cross contaminate the substrate. Always use a clean pipette tip when working with the substrate. Substrate should be protected from light sources.

Stop solution:

- The stop solution is supplied ready to use.
  Note: Stop solution contains 1.5% sodium fluoride. If contact is made with the skin, wash affected area with copious amounts of water.

Sampling Preparation - These should be taken as a guideline only. Modifications of the suggested procedures may be required according to the plant species or tissue tested.

Stem tissue:

- Cut out approximately a 2-inch (5cm) length of stem including the margin of the lesion in the centre of the segment.
- Slice the length of stem transversely through the centre of the stem to expose the pith and cortex.
- Place the processed material into one side of the extraction bag and add 4ml of working strength extraction buffer.
- Use the bottom of a bottle or other blunt object to further crush the sample as much as possible.
- Remove the liquid from the side which is opposite to that which the sample was inserted into.
- The sample is now ready to use.

Root tissue:

- Wash roots gently under running tap water to remove as much soil as possible. Blot the roots with clean paper towels.
- To sample the tap root, cut out a 2-inch (5cm) section of root showing lesions or discoloration
- Place the processed material into one side of the extraction bag and add 4ml of working strength extraction buffer.
- Use the bottom of a bottle or other blunt object to further crush the sample as much as possible.
- Remove the liquid from the side which is opposite to that which the sample was inserted into.
- The sample is now ready to use.

Leaf tissue:

- Select leaves to be tested. If symptoms are present, select symptomatic leaves or portions of leaves for testing.
- Place the material into one side of the extraction bag and add 10ml of working strength extraction buffer.
- Use the bottom of a bottle or other blunt object to further crush the sample as much as possible.
- Remove the liquid from the side which is opposite to that which the sample was inserted into.
- The sample is now ready to use.

Cultured fungi:
Fungi grown in culture may be tested with these kits. Cultured materials may include samples from agar or other solid media, liquid cultures, or materials used for baiting pathogens. Certain cultures of *Phytophthora* spp may not produce strong positive reactions even though infected plant material reacts well; extracts of such cultures can be made reactive by heating.

- For cultures grown on agar media use a cork borer (#5 or similar) to remove a plug containing mycelium from the edge of a colony.
- Place the processed material into one side of the extraction bag and add 4ml of working strength extraction buffer.
- Use the bottom of a bottle or other blunt object to further crush the plug as much as possible and mix the liquid.
- Remove the liquid from the side which is opposite to that which the sample was inserted into.
- The sample is ready to use.

Test Procedure

This kit can be divided into 12, eight assay groups (one strip assays) or can be run as multiple strips, up to a total of 96 assay wells (12 strips).

Note: Four non-sensitised strips are provided and can be included in an assay to monitor for plant background effects.

1 With a pencil number the columns on the lower frosted edge of the strip; this preserves the identity of the strips should they become detached from the frame.

2 Once an assay has started, all steps should be completed without interruption.

   a. Allow all reagents and the wells to reach room temperature (about 30 minutes) before starting the assay.

   b. Prepare the tissue samples and the necessary kit materials.

3 Using a precision micropipette, place 100µl of positive control, negative control and each of the prepared samples into the appropriate micro wells.
Note: Use a separate disposable tip for each pipetting step to avoid cross-contamination.

4 Place the micro well module on the orbital plate shaker and mix for 10 minutes. If a shaker is not available then incubate without shaking at room temperature for at least 30 minutes.

5 At the end of the incubation period, the plate should be washed using a working strength wash buffer previously prepared. The liquid is aspirated from the wells and then refilled with the wash solution until a small meniscus forms at the top of each well. Care should be taken not to overfill the wells. This entire procedure should be repeated a total of 3 times. On the last aspiration the plate should be dried by tapping the plate upside down on several layers of absorbent tissue to remove residual droplets of wash solution.

6 Using a micropipette, place 100µl of antibody-peroxidase conjugate into each micro well.

7 Incubate as in step (4).

8 At the end of the incubation time repeat the wash procedure outlined in step (5). A total of 4 washes should be carried out at this step.

9 Using a micropipette, place 100µl of substrate solution to each well.

10 Incubate as in step (4).

11 At the end of the incubation time add 100µl of stop solution. Mix the micro well module for 10 seconds on an orbital plate shaker. If one is not available then agitate the microwell module by hand.

12 Using a micro plate reader fitted with a 605-650nm filter, measure the absorbance readings in all wells. All readings should be completed within 90 minutes of adding the stop solution.

INTERPRETING RESULTS

- Kits are designed to detect and quantify plant pathogenic fungi in plant tissue and other materials. Infected material and cultures will produce a definite visible colour (**POSITIVE REACTION**) in the wells while reaction wells exposed to non-infected material will remain colourless before the addition of stop solution and will turn pink/red after the addition of stop solution (**NEGATIVE REACTION**).

- The *Phytophthora* spp kit is highly specific for *Phytophthora* spp. And does not react with other genera of fungi, including *Rhizopus*, *Mortierella*, *Rhizoctonia*, *Fusarium*, *Sclerotinia* and most species of *Pythium**.

  The following species of *Phytophthora* have been tested POSITIVE in the *Phytophthora* assay:

boehmeriae	*erythroseptica*
syringae	*cactorum*
fragariae	*drechsleri*
cambivora	*gonapodyides*
palmivora	*cinnamomi*
ilicis	*parasitica*
citricola	*infestans*
citrophthora	*pseudostsugae*
lateralis	*parasitica var. nicotiana*
cryptogea	*megasperma f. sp glycinea*
quininea	*megasperma f. sp. trifolii*
megasperma	*megasperma f. sp. medicaginis*
vignae	

  *Pure culture extracts of *Pythium coloratum* and *P. vexans* show significant cross reactivity in the *Phytophthora* assay. *Pythium aphanidermatum*, *P. dissotochem* and *P. ultimum* may show levels of reactivity in the assay.

PERFORMANCE CHARACTERISTICS

- **Positive Control**: should display an obvious blue colour at the end of the assay indicating that the assay components are performing within specifications.

- **Negative Control**: should appear nearly colourless to the naked eye at the end of the assay and can be used for comparison with sample wells suspected of being slightly positive.

- **Non-sensitised Micro well Strips**: have no antibody and are intended for monitoring non-specific binding effects that may be introduced by the plant material. When these wells are included in an assay they should appear nearly colourless to the naked eye at the end of the assay and should turn ink/red on the addition of stop solution.

Technical Problems

If you have a problem then prompt and comprehensive technical advice is always available from ADGEN Phytodiagnostics at Neogen Europe Ltd.

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