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On-site Plant Pathogen Testing

ISK/STX 92601

Test Characteristics

Test Name Phytophthora Capture Antibody Polyclonal (Rabbit) Catalog Number 92601 **Detection Antibody** Monoclonal (Mouse)

Acronym Phyt Format Lateral Flow Device Diluents SEB1 Genus Phytophthora

Sample Dilution 1:20

Summary

The Phytophthora (Phyt) ImmunoStrip is used to detect the presence of Phytophthora species in many crops including Oak, Potato, and Strawberry. ImmunoStrips are the perfect screening tool for use in the field, greenhouse, and the lab.

Diagnostic Sensitivity

Analytical Sensitivity

True Positives 146 Limit of Detection: 1:5,120 dilution of infected tissue (pathogen titer unknown)

Correct Diagnoses 146

Percent 100%

Analytical Specificity

Inclusivity:

Species Detected1.

Species Detected ¹ :	
Phytophthora alni	Phytophthora alticola-type
Phytophthora asparagi	Phytophthora bisheria
Phytophthora boehmeriae	Phytophthora cactorum
Phytophthora cajani	Phytophthora cambivora
Phytophthora canalensis	Phytophthora capsica
Phytophthora cinnamomi var parvispora	Phytophthora cinnamomi var robiniae
Phytophthora citricola	Phytophthora citrophthora
Phytophthora cryptogea	Phytophthora drechsleri
Phytophthora erythroseptica	Phytophthora europaea
Phytophthora fragariae var fragariae	Phytophthora fragariae var rubi
Phytophthora glovera	Phytophthora gonapodyides
Phytophthora heveae	Phytophthora hibernalis
Phytophthora infestans	Phytophthora kernoviae
Phytophthora lagoariana	Phytophthora lateralis
Phytophthora lavandula	Phytophthora meadii
Phytophthora medicaginis	Phytophthora megasperma
Phytophthora melonis	Phytophthora nemorosa
Phytophthora nicotianae	Phytophthora niederhauserii
Phytophthora palmivora	Phytophthora parasitica
Phytophthora pistaciae	Phytophthora porri
Phytophthora pseudotsuga	Phytophthora quercina

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Species Detected1:

Phytophthora ramorum	Phytophthora richardiae
Phytophthora sinensis	Phytophthora siskiyouensis
Phytophthora sojae	Phytophthora syringae
Phytophthora taxon Agatis (PTA)	Phytophthora tropicalis
Phytophthora uliginosa	

¹The list above represents the Phytophthora species that have been shown to be detected by the Phytophthora genus ImmunoStrips test and does not represent all species that may be detected. If you have confirmed detection of a Phytophthora species not on this list, please contact us. We would like to work with you to further validate the Phytophthora genus ImmunoStrip detection capabilities.

Exclusivity:

Cross-reacts With:

Phytopythium litorale	Plasmopara halstedii
Plasmopara viburni	Pythium aphanidermatum
Pythium heterothalicum	Pythium paroecandrum
Pythium sylvaticum	Pythium vanterpoolii

Does Not Cross-react With:

Pythium amazonicum	Pythium arrhenomanes
Pythium catenulatum	Pythium graminicola
Pythium hydnosporum	Pythium irregulare
Pythium myriotilum	Pythium myriotylum
Pythium olegandrom	Pythium olegandrum
Pythium paroecandrum	Pythium radicrenses
Pythium splendes	Pythium ultimum
Pythium ultimum var. ultimum	Pythium vexans type

Diagnostic Specificity

True Negatives 79
Correct Diagnoses 79

Percent 100%

Selectivity:

- Ciccurry.			
No Matrix Effect Observed With:			
Arctostaphylos leaves	Banana midrib	Black walnut leaves	Blackberry leaves
Bougainvillea leaves	Camellia leaves	Cantaloupe leaves	Cinnamomum leaves
Citrus leaves	Cowpea leaves	Cowpea roots	Cucumber leaves
Ficus leaves	Garlic leaves	Garlic roots	Garlic stem
Grape leaves	Hops leaves	Lilac leaves	Lonicera leaves
Loropetalum leaves	Magnolia leaves	Nerium leaves	Pepper leaves
Pepper roots	Photinia leaves	Pine needles	Potato leaves
Potato tubers	Pyracantha leaves	Quercus leaves	Raspberry leaves
Rhododendron leaves	Rosa leaves	Sequoia leaves	Soybean cotyledons
Soybean leaves	Soybean roots	Squash leaves	Strawberry leaves
Syringa leaves	Tomatillo leaves	Tomato leaves	Tomato roots
Umbellularia leaves	Viburnum leaves	White Oak leaves	

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USER GUIDE: Phytophthora ImmunoStrip® (Product Number 92601)

KIT INFORMATION

Intended Use

The *Phytophthora* ImmunoStrip is designed for on-site testing of plant tissues suspected of infection with *Phytophthora* species. The test is recommended for use as a preliminary screening tool in survey programs for *Phytophthora* species such as *P. ramorum* (Sudden Oak Death) and *P. kernoviae*. The test is also suitable for detection of *Phytophthora* species that affect other important crops such as *P. fragariae* in strawberry or *P. infestans* in potato. A complete list of detectable *Phytophthora* species can be found on page 2 of this document.

Storage of Kit

ImmunoStrips should be stored refrigerated (2 - 8 °C) between uses and tightly sealed in the desiccated container at all times.

ImmunoStrips and extraction buffer should be warmed to room temperature (18 - 30 °C) prior to use.

ImmunoStrip Kit (ISK) Includes

- ImmunoStrips
- SEB1 sample extraction bags
- User guide

ImmunoStrips (STX) purchased separately do not include buffer filled mesh bags.

What's required to perform the assay?

- Scissors, knife or razor blade.
- SEB1 sample extraction buffer
- Sample extraction equipment (e.g., Agdia sample extraction bags; Agdia tissue homogenizer - ACC 00900 or blunt object such as a pen or marker)
- Letter holder or other device to hold sample extraction bags.

PERFORMING THE ASSAY (*Special Attention Required)

Prepare Sample



Samples should be taken from symptomatic plant tissue when possible. Agdia sample extract bags contain 3 mL of extraction buffer, requiring 0.15 g tissue for the optimal 1:20 dilution. For most samples, an approximate sample size of 2.5 cm² or 1 inch² is adequate; however, thick or dense tissues can alter the targeted 1:20 dilution. Extraction and testing of overly degraded, dried, or large amounts of tissue can cause erroneous results.

Note: It is recommended that you use a clean cutting tool for each sample. If you must reuse the cutting tool, first wipe off the cutting edge and disinfect in a 10% bleach solution before cutting into a new sample.

Cut open the sample extraction bag along the top of the label. Be careful not to spill the buffer.

*SEB1 Buffer is required to perform this assay.



Insert the sample between the mesh linings near the bottom of the sample extraction bag.



Extract the sample by thoroughly macerating it with an Agdia tissue homogenizer or a blunt object such as a pen or marker.

An adequately extracted sample will result in a homogenous green or light brown colored solution. Allow the resulting solution to settle for 3 minutes before inserting the ImmunoStrip.

Perform Assay



Remove an ImmunoStrip then reclose the container. When handling the strips, always grasp the top of the strip marked with the test name. Do not remove the protective covering.

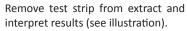
Insert the sample end of ImmunoStrip into the bag until submerged in the extract up to the white line. For best results, insert the ImmunoStrip into the channel portion of the bag (no mesh). Do not allow the side of the ImmunoStrip to come into contact with foam or bubbles (if present). Trimming the bag may also allow for more control when inserting the ImmunoStrip into the bag.

*Be sure to insert the "sample" end of the strip no more than ¼ inch or to the white line on the ImmunoStrip label.



Place the bag in a letter holder or other device in upright position. Allow the ImmunoStrip test to remain in the sample extract for 30 minutes. Positive results may be visible in as little as 5 minutes. Lower titer samples may take up to 30 minutes.

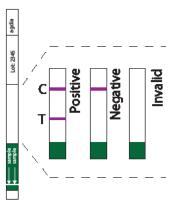
Interpret Results



If the control line (C) is visible and the test line (T) is also present at any intensity of pink/purple, this indicates a positive** result.

If only the control line (C) is visible, this indicates a negative result.

If no lines are present, the test is invalid (see troubleshooting).



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Performance Information *

TABLE 1 represents a list of *Phytophthora* species that are detected by the *Phytophthora* ImmunoStrip.

Phytophthora Species Tested			
P. alticola-type	P. citrophthora	P. lateralis	P. quercina
P. asparagi	P. cryptogea	P. lavandula	P. ramorum
P. alni	P. drechsleri	P. meadii	P. richardiae
P. boehmeriae	P. europaea	P. medicaginis	P. taxon Agatis (PTA)
P. bisheria	P. erythroseptica	P. megasperma	P. sinensis
P. cambivora	P. fragariae var. fragariae	P. melonis	P. siskiyouensis
P. cactorum	P. fragariae var. rubi	P. nemorosa	P. sojae
P. cajani	P. gonapodyides	P. nicotianae	P. syringae
P. canalensis	P. glovera	P. niederhauserii	P. tentaculata
P. capsici	P. heveae	P. palmivora	P. tropicalis
P. cinnamomi	P. hibernalis	P. parasitica	P. uliginosa
P. cinnamomi var. parvispora	P. infestans (6 isolates)	P. pistaciae	
P. cinnamomi var. robiniae	P. kernoviae	P. porri	
P. citricola	P. lagoariana	P. pseudotsuga	

^{*} This test is known to cross-react with the following species of *Pythium: P. sylvaticum, P. paroecandrum, P. heterothalicum, P. aphanidermatum* and *P. vanterpoolii.*

Safety

ImmunoStrips and sample extraction buffer are non-hazardous.

Troubleshooting

Control line did not develop.	This situation is generally caused by over-submergence of the test strip in the sample extract.
	Also, ImmunoStrips inserted immediately after extraction and prior to settling for three minutes have an increased chance of device failure due to the possibility of liquid wicking in above the sample line.
	If no control line is present, results should be considered invalid, and the test should be repeated.
Test runs very slow or not at all.	This can be caused by using too much tissue for extraction. Repeat the test using less tissue or by further diluting your previous sample extract 1:10 with SEB1.
	If the above is not the case, make sure the test components were warmed to room temperature before use and are within their expiration date.
Test has a green or pigmented test line.	This can be caused by using too much tissue for extraction. Repeat the test using less tissue or by further diluting your previous sample extract 1:10 with SEB1.
	**In rare cases, the tissue type may cause a pigmented line. Green lines should not be considered a positive result. Red, orange, or purple fruits and tissues (for example, red begonia leaves) may cause what appears to be a positive test line. It is recommended that you contact Agdia before testing these types of samples.
Test and / or control line is weak.	Make sure the test is within its expiration date.
	If kit contents were left open too long, the strips could have absorbed moisture, which can affect test results. Be sure to always keep the ImmunoStrip vial tightly sealed between uses.
	The test line may be weak due to low pathogen titer in the sample.

Alternate Protocol(s)

Alternate protocols for culture testing can be found on Agdia's website.

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