



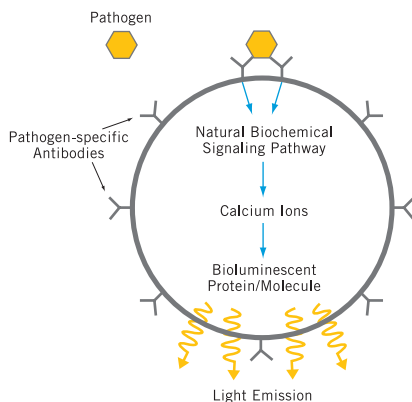
Phytophthora Zephyr Assay

The CANARY®- Zephyr System delivers rapid, sensitive and specific detection of *Phytophthora* in an easy to use format.

CANARY® technology was developed by MIT-Lincoln Laboratory under a DARPA contract, and commercialized by PathSensors, Inc. The technology utilizes biosensors, which express surface-bound, target-specific antibodies and a bioluminescent protein. When the biosensor binds to its target pathogen, the antibodies trigger the intracellular release of calcium. This calcium causes the bioluminescent protein to emit light. Sophisticated algorithms analyze this light output, resulting in definitive “positive” or “negative” test results.

Advantages of this system are its extreme speed and sensitivity. The speed of detection is a result of rapid intracellular signaling. The sensitivity is achieved through signal amplification within the biosensor. This leading edge technology identifies *Phytophthora* in < 20 minutes.

CANARY® Biosensor



Assay Specifications

Biosensor Analytical Sensitivity	200 ng (wet weight) of <i>P. ramorum</i> culture at 1 µg/mL
Assay Specificity	1% of <i>Phytophthora ramorum</i> infected rhododendron leaves
Cross-Reactivity	No cross-reactivity with <i>Pythium</i>
Time to Results	< 20 minutes



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MADE IN THE USA

Testing Protocol:

- Process leaf samples in Extraction Buffer
- ▼
- Centrifuge sample for 1 minute
- ▼
- Transfer 100 µL extract to Capture Tube with Capture Beads
- ▼
- Add Extraction Buffer
- ▼
- Rotate for 10 minutes
- ▼
- Centrifuge sample for 1 minute
- ▼
- Retrieve beads on magnetic rack for 2 minutes and remove supernatant
- ▼
- Wash and resuspend beads in Sample Diluent
- ▼
- Transfer 200 µL of sample to Assay Tube
- ▼
- Add Biosensors
- ▼
- Centrifuge 5 seconds
- ▼
- Place Assay Tube in Luminometer (read for 1 minute)
- ▼
- RETRIEVE RESULTS**

Testing and Results:

Specificity		
<i>Phytophthora</i>	<i>Pythium</i>	Fungi
Tested positive at 100 µg/mL of pure culture	Tested negative at 100 µg/mL of pure culture	Tested negative at 1 mg/mL of pure culture
<i>Phytophthora cactorum</i> <i>Phytophthora cambivora</i> <i>Phytophthora capsici</i> <i>Phytophthora cinnamomi</i> <i>Phytophthora citrophthora</i> <i>Phytophthora kernoviae</i> <i>Phytophthora infestans</i> <i>Phytophthora nicotianae</i> <i>Phytophthora niederhauserii</i> <i>Phytophthora ramorum</i> <i>Phytophthora sojae</i>	<i>Pythium amasculinum</i> <i>Pythium aphanidermatum</i> <i>Pythium graminicola</i> <i>Pythium helicandrum</i> <i>Pythium myriotylum</i> <i>Pythium inflatum</i> <i>Pythium irregulare</i> <i>Pythium spinosum</i> <i>Pythium sylvaticum</i> <i>Pythium ultimum</i>	<i>Fusarium oxysporum</i> <i>Mortierella elongata</i> <i>Sclerotinia sclerotiorum</i> <i>Trichoderma harzianum</i>

Phytophthora Assay Statistics	
Positive Predictive Value	100%
Negative Predictive Value	> 98%
Sensitivity	> 98%
Specificity	100%

Phytophthora Assay Kit Configuration (For 24 assays):	
<i>Ph</i> Biosensor Reagent (A)	1 tube, 1 mL each
Biosensor Resuspension Buffer (B)	1 barcode labeled tube
Reconstitution Tube	1 tube
Negative Control (NC)	1 tube
Positive Control (PC)	1 tube
Extraction Buffer (X)	2 bottles, 45 mL each
Sample Diluent (D)	2 bottles, 45 mL each
Capture Beads (E)	3 tubes, 50 µL each
Capture Tubes and Assay Tubes	50 tubes each



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