Validation Report: ELISA

PSA/SRA 20501 • *Impatiens necrotic spot virus* (INSV)

Test Characteristics

Test Name	Impatiens necrotic spot virus	**Capture Antibody**	Monoclonal (Mouse)
Catalog Number	20501	**Detection Antibody**	Monoclonal (Mouse)
Acronym	INSV	**Format**	Compound ELISA
Genus	Orthotospovirus	**Diluents**	GEB/ECI
		Sample Dilution	1:10

Summary

This ELISA test is a qualitative serological assay for the detection of Impatiens necrotic spot virus (INSV) in fruit, ornamental, and vegetable leaves. INSV is a member of the Orthotospovirus genus known for their enveloped, spherical-shaped virus particles.

Diagnostic Sensitivity

True Positives	29
Correct Diagnoses	29
Percent	100%

Analytical Sensitivity

Limit of Detection: 1:12,960 dilution of infected tissue (pathogen titer unknown)

Analytical Specificity

Inclusivity:

This assay was designed to detect all strains and isolates of INSV. Twenty distinct samples of INSV have been experimentally proven to be detected.

Exclusivity:

Cross-reacts With:

None known	

Does Not Cross-react With:

Capsicum chlorosis virus (CaCV)	Chrysanthemum stem necrosis virus (CSNV)
Groundnut bud necrosis virus (GBNV)	Groundnut ringspot virus (GRSV)
Iris yellow spot virus (IYSV)	Soybean vein necrosis virus (SVNV)
Tomato chlorotic spot virus (TCSV)	Tomato spotted wilt virus (TSWV)
Watermelon silver mottle virus (WSMoV)	

India Contact:
Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034 (INDIA). Ph: +91-11-42208000, 42208111, 42208222, Mobile: +91-9810521400, Fax: +91-11-42208444
Email: customerservice@lifetechindia.com Website: www.lifetechindia.com

Diagnostic Specificity

True Negatives	179
Correct Diagnoses	179
Percent	100%

Selectivity:

No Matrix Effect Observed With:			
Alstroemeria leaves	Alternanthera leaves	Angelonia leaves	Anemone leaves
Argranthemum leaves	Artichoke leaves	Antirhinum leaves	Aster leaves
Astilbe leaves	Bacopa leaves	Bean leaves	Begonia leaves
Beet roots	Blackberry leaves	Blueberry leaves	Browallia leaves
Buddleia leaves	Calibrachoa leaves	Campanula leaves	Chrysanthemum leaves
Cleome leaves	Coleus leaves	Coreopsis leaves	Cucumber leaves
Cyclamen perscum leaves	Cymbidium leaves	Dahlia leaves	Diascia leaves
Dianthus leaves	Fuchsia leaves	Gaillardia leaves	Garlic leaves
Gerbera leaves	Helichrysum leaves	Hosta leaves	Hydrangea leaves
Impatiens leaves	Ipomoea leaves	Kalanchoe leaves	Lamium leaves
Lettuce leaves	Limonium Statice leaves	Lobelia leaves	Mimulus leaves
Nandina leaves	Nemesia leaves	Nepeta leaves	New Guinea Impatiens leaves
Osteospermum leaves	Papaya leaves	Peanut leaves	Pepper leaves
Penstemon leaves	Petunia leaves	Phlox leaves	Portulaca leaves
Potato leaves	Primrose leaves	Radicchio leaves	Ranunculus leaves
Rose leaves	Rubus leaves	Salvia leaves	Scabiosa leaves
Scaevola leaves	Soybean leaves	Spinach leaves	Strawberry leaves
Strawflower leaves	Tobacco leaves	Tomato leaves	Torenia catalina leaves
Verbena leaves	Verbascum leaves	Watermelon leaves	

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User Guide: Compound-ELISA Reagent Set

General User Guide • GEB / ECI • Alkaline Phosphatase

Test Principle, Intended Use and Limitations

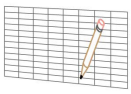
This product is intended for the qualitative detection of the target analyte via a direct, triple antibody sandwich protocol known as Compound-ELISA. Upon successful completion of the test, samples containing the target analyte will turn yellow, due to the alkaline phosphatase enzyme label, while negatives will remain colorless. Visit the product webpage for information regarding host reactions, cross-reactions, alternate protocols, 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 00111. Do not store user-prepared 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.

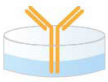


Test Preparation

Scan for buffer formulations

1. Visit the product webpage to view buffer formulations, 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.

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 and Plate Loading

1. Sample symptomatic tissue if possible. Other plant parts may be tested, including asymptomatic tissue.
2. At the time of testing, grind and dilute the samples at a 1:10 ratio with GEB.

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

3. Empty coated plate contents and wash 3 times with 1X PBST.
4. Tap plate dry using lint-free paper towel.
5. Dispense 100 µL of the extracted samples, positive control, negative control, and GEB into the plate following your logsheet.
6. Incubate plate in the humid box for either 2 hours at room temperature or overnight at 2 - 8 °C.

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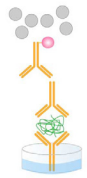
Prepare Detection Solution

1. Prepare the mix of the detection antibody (Bottle A) and enzyme conjugate (Bottle B) in a non-binding container, such as Agdia's sample cups (ACC 00960).
2. Dilute both the thoroughly-mixed Bottle A and Bottle B, per the dilution on the labels, in 1X ECI buffer (see example). You will need 100 µL of diluted detection solution per well; a full plate will need 10 mL.

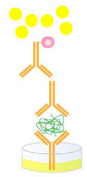
   Example: (Wells Used 16 x 100 µL) ÷ 200[†] = 8[‡] µL Bottle A and Bottle B
   [†]Bottle dilution will be either 100 or 200
   [‡]Add 8 µL of both Bottle A and Bottle B into 1X ECI
3. Wash the sample from the plate 8 times using 1X PBST.
4. Tap plate dry using lint-free paper towel.
5. Thoroughly mix and pipette 100 µL of the diluted detection solution into each testwell.
6. Incubate plate in the humid box for 2 hours at room temperature.

Prepare Substrate



1. Add 1 PNP substrate tablet per 5 mL of 1X PNP substrate buffer into a dedicated container and keep in the dark until use. You will need 100 µL of diluted PNP solution per well; a full plate will need 10 mL. Ensure tablets are dissolved before use.
2. Wash the detection solution from the plate 8 times using 1X PBST.
3. Tap plate dry using lint-free paper towel.
4. Pipette 100 µL of dissolved PNP solution into each testwell.
5. Incubate, protected from light, for 1 hour at room temperature.

Interpreting Results



1. Visually inspect wells and remove bubbles, if present. Measure O.D. values with a spectrophotometer at 405 nm or 405 nm with a 650 nm blank.
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.*	*0.105*	*2 x Healthy Avg.*	*0.210*	
	Sample 1	*0.355 (Positive)*	*Sample 2*	*0.190 (Negative)*	

Method 2	*Healthy Avg.*	*0.105*	*Std. Dev.*	*0.030*	*Healthy Avg. + 3 x Std. Dev.*	*0.195*
	Sample 1	*0.355 (Positive)*	*Sample 2*	*0.190 (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.

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