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# INSTRUCTIONS FOR USE

**Pseudomonas syringae One-Pot Starter Detection Kit** **Cat No: PS01-K199**

## Intended Use

To enable point-of-care testing for the immediate detection of *Pseudomonas syringae* in plant samples. To maximize harvests, preserve resources, maintain crop purity.

## Introduction

Testing kit designed for early rapid detection of pathogens in plants. The kit is self sufficient, so there is no need for the end user to send samples elsewhere for analysis. The kit is designed to work with an instrument (CRISPOT Detection Device, Cat# CSP01-V19A, SOLD SEPARATELY), which screens for the presence of a portion of the pathogen's genome in all samples, thus identifying infected plants. It is important for the end-user to label the samples according to the plants they were extracted from to avoid confusion. The user is expected to spend around 30 minutes on sample preparation and setting up the device. Once set-up is complete, running the test on the detection device will take 25 minutes.

## Disclosure

This product is not to be used as a drug, medical device, food additive, cosmetic, or household chemical. It is not to be used in therapeutic, consumer, or pesticidal applications. Not for resale.

**Intended for users who have already purchased the CRISPOT Detection Device** (Cat# CSP01-V19A) and who are carrying out the tests for the first time.

## Starter Kit Components

| No | Component | Amount |
|---|---|---|
| 1 | Tube Rack | 1 |
| 2 | Magnetic Rack | 1 |
| 3 | 200 µL Pipette | 1 |
| 4 | 25 µL Pipette | 1 |
| 5 | Pipette Tips | 170 |
| 6 | Grommets | 16 |
| 7 | PCR tube strips with Lysis Buffer | 2 (8-well) red strip tubes |
| 8 | PCR tube strips with Buffer-Bead Mix | 2 (8-well) strip tubes |
| 9 | Wash Buffer A | 7 mL |
| 10 | Wash Buffer B | 7 mL |
| 11 | Elution Buffer | 750 µL |
| 12 | PCR tube strips with Reaction Buffer | 2 (8-well) blue strip tubes |
| 13 | PCR tube strips with Master Mix | 2 (8-well) strip tubes |
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**Not Provided**

- Gloves
- Sterile pipette filter tips (not included with test kits, available as a separate add-on)

## Kit Storage

Store all kit components at 2-25°C for up to 6 months.

The premixtures in the PCR tubes (component 13) should be protected from light. The foil bag with desiccant offers light and moisture protection as long as the bag is tightly closed. Always keep the strips in the bag when not in use, as exposure to light can cause deterioration and lead to inaccurate results.

## Precautions

- Ensure the working environment is clean before starting the experiment.
- Always wear a new pair of examination gloves when handling samples and performing the assay.
- Remove the appropriate number for tubes from the strip tubes containing the buffers and pre-mix based on the number of samples being tested.
- The presence of inhibitors can lead to invalid or false-negative results.
- Mutations in the target regions of the *Pseudomonas syringae* genome, covered by the primers in this kit, may prevent the detection of the pathogen.
- Adhere to good laboratory practices to ensure proper use of this kit. Keep both the kit and reactions free from contamination. Carefully inspect all reagents for signs of contamination, and do not use any reagents that appear contaminated.

## Protocol

1. Using the provided clean grommet (6), collect plant tissue from areas with suspected infection. Collect either two stem pieces, two petiole pieces, or four leaf pieces, and place them directly into a red tube containing lysis buffer (7). Close the lid securely.
    a. Folding or stacking of layers of leaves can be done to save time.
    b. Do no exceed four discs, as the purification process can be overloaded.
2. Incubate the tube with leaf discs and lysis buffer in the CRISPOT device using the heating program.
    a. Using the top and middle button of the portable device, select the heating program.
    b. Start the program by pressing the bottom button.
3. Transfer the entire volume of lysis buffer into a tube containing the Buffer-Bead Mix (8) using the 200 µL pipette (3). Mix thoroughly by pipetting up and down several times, then incubate in the CRSIPOT device using the heating program.
4. Place the tube on the magnetic rack (2) to allow the beads to collect next to the magnet.
5. Remove the buffer without disturbing the pellet of beads using the 200 µL Pipette (3).
6. Remove the tube from the magnetic rack (2) and resuspend the beads in Wash Buffer A (9) using the 200 µL pipette (3).
7. Repeat steps 4 to 6 again for a second wash with Wash Buffer A (9).

8. Repeat steps 4 to 7 using Wash Buffer B (10), for a total of 4 washes (2 using Wash Buffer A and 2 with Wash Buffer B).
9. For the final wash, leave the tube on the magnetic rack (2) and use the 200 µL pipette (3) to remove any remaining wash buffer without disturbing the beads.
10. Allow the beads to dry at room temperature for 5 minutes, uncovered, on the magnetic rack (2).
    a. Ensure as much wash buffer as possible is removed, as any residual wash buffer can inhibit the reaction and lead to false negatives.
    b. Do not allow the beads to over-dry, as this may make it difficult to elute the target material.
11. Remove the tube from the magnetic rack (2) and resuspend the beads in Elution Buffer (11) using the 25 µL pipette (4).
12. Incubate the tube in the CRISPOT device using the heating program.
13. Place the tube on the magnetic rack (2) to allow magnetic beads to collect next to the magnet.
14. Using the 25 µL pipette (4), transfer the entire liquid into a new tube with Reaction Buffer (12), avoiding disturbance of the beads.
15. Mix thoroughly by pipetting up and down using the 25 µL pipette (4) and transfer 25 µL of the diluted reaction buffer into a tube containing Master Mix (13).
16. Mix the solution by pipetting up and down using the 25 µL pipette (4). Close the lid and place into the CRISPOT machine. Run the test using the testing program.
17. After the run is completed, the *Pseudomonas syringae* infection status of each sample will be displayed: red for positive and green for negative.

## Warning

- Ensure all plant material is fully submerged in the lysis buffer before incubating in the CRISPOT device.
- Change gloves and grommets between each sampling to avoid cross-contamination.
- Use a new pipette tip for each sample transfer or wash to prevent cross-contamination.
- Dispose of used materials in the regular trash.