AmplifyRP® XRT+ for *Xf*
Rapid DNA Amplification Test Kit
Product No. XCS 34503

Intended Use:
AmplifyRP XRT+ for *Xf* is a rapid DNA amplification and detection platform designed for end-point or real-time detection of *Xylella fastidiosa* in almond, blackberry, blueberry, citrus, grapevine, and olive. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify *Xf* DNA at a single operating temperature (42°C).

SPECIFICITY: Detects only *Xf.* Does not cross-react with other closely related bacteria.
SENSITIVITY: 22 copies of Xf genomic DNA in crude petiole extract (1:10 w/v).
PROBE LABEL: FAM (Agdia has optimized this kit for use with the AmpliFire® manufactured by Agdia, Inc. Contact us for information on use with other instruments.) Alternatively, amplicons may be detected using an Amplicon Detection Chamber (sold separately).

Contents of Kit:
- Reaction pellets
- Pellet Diluent
- 1.5 mL microcentrifuge tubes
- AMP1 extraction buffer
- Sample extraction bags, mesh

Not Included but Required:
- See table on page 4.

Kit Storage:
All kit components should be stored refrigerated (2 - 8 °C).

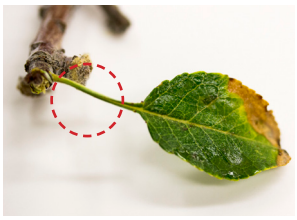
Before use, allow all kit components to warm to room temperature (18 - 30 °C) for 20 to 30 minutes.

NOTE: AmplifyRP is a very sensitive molecular assay. Do not re-use disposable kit components. It is recommended that latex gloves be worn when taking samples and performing assay. If wearing latex gloves, change them between samples and test runs. Sanitize work area and non-disposable equipment between runs with bleach solution that has a concentration of at least 600 ppm (1:10 of household bleach solution).

Prior to setting up reactions, turn on the AmpliFire (or an alternative isothermal instrument/heat block) so that it is ready to accept reactions. It should be pre-heated to the recommended 42 °C before inserting reactions and setup to run on the FAM channel.

Sample Preparation

1. *Xf* bacteria is most often not evenly distributed throughout an infected plant's vascular system. We recommend testing petiole and/or stem tissue from multiple areas of the plant showing signs of infection.

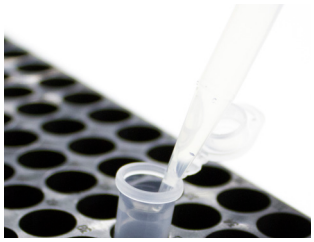


Petiole from symptomatic almond leaf

2. Select 0.3 g of suspect tissue and Insert the sample between the mesh linings of the sample extraction bag. Add 3.0 mL of AMP1 extraction buffer to the tissue inside the bag. Extract the tissue by thoroughly macerating it with a blunt object such as a pen. For maximum sensitivity, allow the extract to incubate for 30 minutes at room temperature before testing.

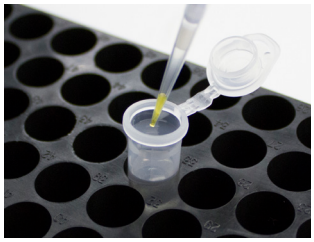
**Note:* This test was optimized using a 1:10 tissue to buffer ratio for sample extraction

3. Pipette 1 mL of PD1 (Pellet Diluent) into a 1.5 mL microcentrifuge tube for each sample being tested.



4. Transfer 10 µL of sample extract into the microcentrifuge tube containing PD1 diluent and **mix well.**

Your samples are now ready to be tested.



NEXT STEP - DETECTION:

page 2. Real-Time Detection via AmpliFire

page 3. End-Point Detection via Amplicon Detection Chamber

Click here to access your lot specific QR code

Test Protocol for Real-Time Detection In AmpliFire®

1. Press the “Execute Reaction” button on the AmpliFire®. Then press “Scan Product Code”.

2. Scan the barcode found by following the hyperlink on page 1. The barcode scanner is located on the left side of the AmpliFire.

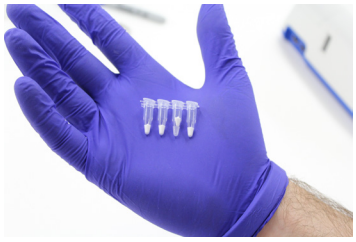
Note: Scanning works best when the barcode is held 3 - 4 inches from the scanner in an area with sufficient ambient light.

Once the AmpliFire has accepted the scan and displayed run method, click “Next”.

3. Follow on-screen prompts to name your reaction and individual sample IDs.

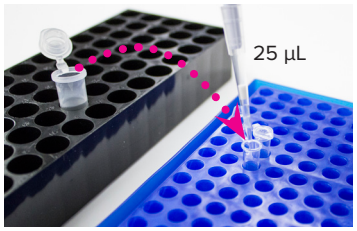
Sample IDs for individual wells are optional. If you prefer to use the default values, click “FINISH”.

4. Remove a canister of reaction pellets from the white foil pouch labeled with the barcode. Then remove a strip of reaction pellets from the desiccated container. While securing the strip of pellets in a 200 µL PCR tube rack, cut the number of reaction pellets from the strip that are intended for use.



Reaction Pellets are light sensitive. Immediately place remaining reaction pellets back into the desiccated tube and then insert the desiccant tube into the foil pouch to protect from light.

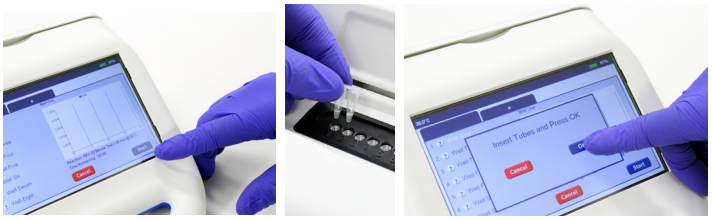
5. Transfer 25 µL from the microcentrifuge tube containing your diluted sample extract into the reaction pellet (clear tube).



Tightly recap the reaction tube. Mix well and centrifuge. If you cannot vortex the reaction, mix by gently flicking the side of the tube. If you do not have a centrifuge available, you may manually shake the liquid to the bottom of the reaction tube.

IMPORTANT: DO NOT TRANSFER MORE THAN THE PRESCRIBED 25 µL DURING THIS STEP! IMMEDIATELY PROCEED TO THE NEXT STEP ONCE THE REACTION HAS BEEN REHYDRATED.

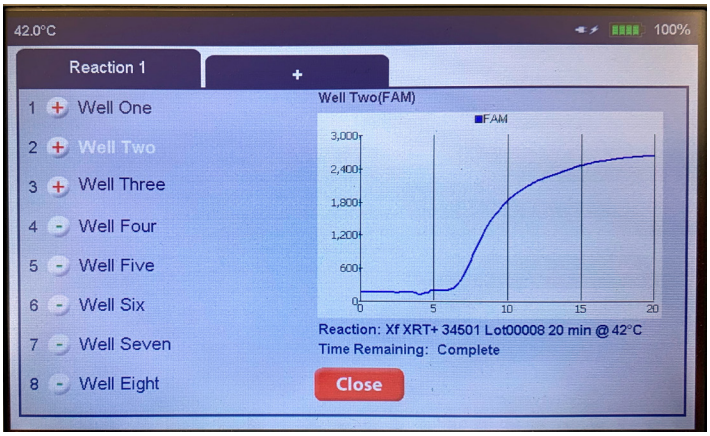
6. Press “Start” on the AmpliFire. Immediately follow the prompts to add your reactions, press “OK”, and put the lid down.



7. After 4 minutes of incubation remove the reaction(s) from the AmpliFire. Quickly mix, spin, and reinsert the reaction(s) into the AmpliFire to continue monitoring results. Take care to ensure the tubes are in their original positions and orientations.

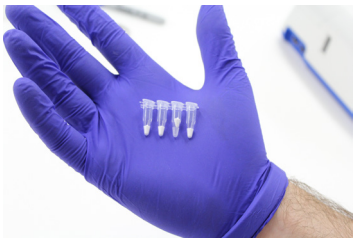
8. After 20 minutes of total run time the instrument will beep, indicating the test is complete. The test results will be visible next to the well designation on the screen, and should be interpreted as follows:

(+) = Positive for *Xf* (-) = *Xf* not detected (!) = Invalid



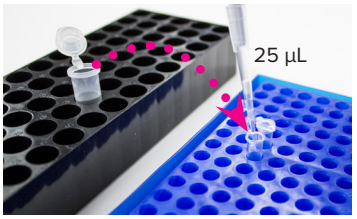
Test Protocol for End-point Detection Using Heat Block/Amplicon Detection Chambers

Amplification



1. Allow heat block to warm to 42 °C before preparing reactions. If using an Agdia-supplied heat block, allow 2 to 3 minutes for this step.

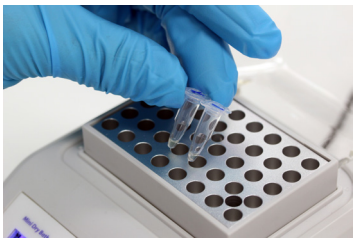
2. Remove the strip of reaction pellets from the desiccated container included in the kit. While securing the strip of pellets in a 200 µL PCR tube rack, cut the number of reaction pellets from the strip that are intended for use. Immediately place remaining reaction pellets back into the desiccated tube for later use.



3. Transfer 25 µL from the microcentrifuge tube containing your sample extract into the reaction pellet (clear tube).

   Tightly recap the reaction tube. Mix well and centrifuge. If you cannot vortex the reaction, mix by gently flicking the side of the tube. If you do not have a centrifuge available, you may manually shake the liquid to the bottom of the reaction tube.

   IMPORTANT: DO NOT TRANSFER MORE THAN THE PRESCRIBED 25 µL DURING THIS STEP! IMMEDIATELY PROCEED TO THE NEXT STEP ONCE THE REACTION HAS BEEN REHYDRATED.



4. Add reaction to the portable heat block for 4 minutes. After 4 minutes of incubation remove the reaction from the heat block. Quickly mix, spin, and reinsert the reaction into the heat block for an additional 16 minutes.

5. Immediately remove reaction from heat block and proceed to detection steps.

Detection

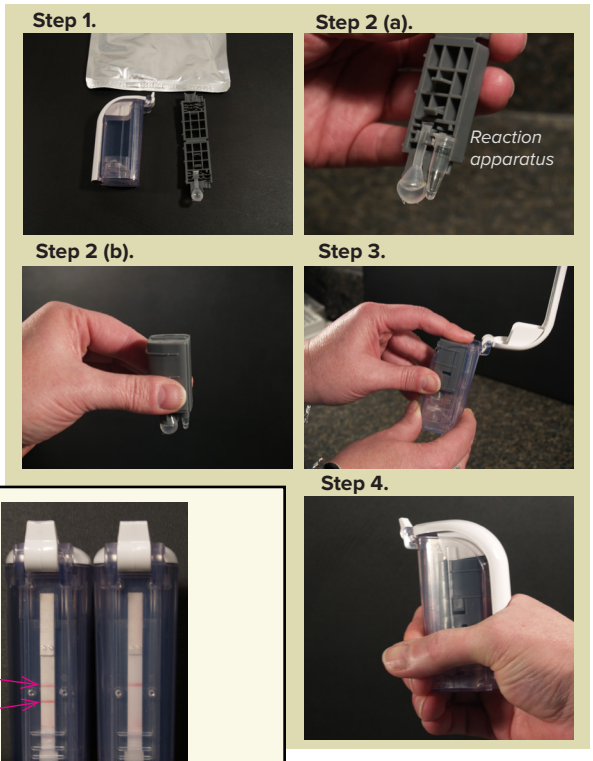
In order to avoid possible contamination of future tests, DO NOT open the reaction pellet.

1. Open the foil pouch containing the Amplicon Detection Chamber (ADC). There are two pieces to the chamber as indicated in the figure to the right.

2. **a.)** Add the unopened reaction tube to reaction apparatus as illustrated to the right. **b.)** Once the tube has been added, snap the apparatus shut which will immobilize the reaction tube.

3. Add the reaction apparatus to the detection chamber housing as indicated. **IMPORTANT: The reaction tube should be facing toward the lateral flow strip, contained in the housing, during this step.**

4. Push down on the handle of the detection chamber housing until it snaps shut. Wait 20 minutes before interpreting results. Positive results may be visible in as little as 5 to 10 minutes. Samples that contain lower copy numbers may take up to 20 minutes to produce a positive test line.



Interpret Results

Result	Lateral Flow Strip Reaction
Positive	Control and Test lines are both visible.
Negative	Control line is visible. Test line not visible.
Invalid	Control line not visible.

Limitations

The following is a description of factors that could limit test performance or interfere with proper test results.

Reaction Volume: Care should be taken to ensure the volume used to rehydrate the reaction is within +/- 10 % of the prescribed 25 µL mentioned in step 5 and 3 the Test Protocols. Deviating outside this tolerance may result in test failure.

Inhibition: Some plant species may cause inhibition with this assay if the sample extract is too concentrated. Please follow the extraction and sample dilution steps carefully.

Addition of sample extract to reaction pellet: It is important to add only the prescribed amount of sample extract to the pellet diluent tubes. Adding too much extract may cause test failure.

Storage: Test results may be weak or the test may fail if the storage instructions are not followed properly. The lyophilized test components must remain protected from light to prevent bleaching and sealed with desiccant when not in use to prevent moisture degradation, which may affect test results. Do not store pellets at temperatures greater than 42 °C, even for short periods of time, as this may cause test failure.

Questions or Technical Support:

Phone: 800-622-4342 (toll-free) or 574-264-2014
Fax: 574-264-2153
E-mail: info@agdia.com for sales and general product information
techsupport@agdia.com for technical information and troubleshooting

Web: www.agdia.com

Accessory Items Not Included In The Kit:

Below are accessories you may consider purchasing depending on which detection method you elect to utilize for this kit. In addition to the items below you will need pipettes, and corresponding tips, capable of accurately dispensing 5 µL, 25 µL, and 1 mL volumes.

REAL-TIME DETECTION

This assay was designed for use with the AmpliFire® Isothermal Fluorometer which can be purchased by ordering the item number below.

Item Number	Description
AFR 60400	AmpliFire® Isothermal Fluorometer

END-POINT DETECTION

The items listed below are required if you plan to utilize the end-point detection option with this assay.

Item Number	Description
ADC 98800/0001	Amplicon Detection Chamber
ACC 00150	AmplifyRP Acceler8® Starter Pack • Portable heat block • 5 µL, 10µL, and 25 µL mini pipettes • 0 - 100 µL aerosol pipette tips (96 count) • 200 µL PCR tube rack