AmplifyRP® XRT+ for *Cms*
Rapid DNA Amplification Test Kit
Product No. XCS 70002

Intended Use:
AmplifyRP XRT+ for *Cms* is a rapid DNA amplification and detection platform designed for end-point or real-time detection of *Clavibacter michiganensis* subsp. *sepedonicus* in potato tubers and bacterial culture. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify *Cms* DNA at a single operating temperature (39 °C).

SPECIFICITY: Detects only *Cms*. Does not cross-react with other *Clavibacter* species.
SENSITIVITY: 0.5 pg of *Cms* DNA
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
- 100 µL Pellet Diluent Tubes
- AMP1 extraction buffer

Not Included but Required:
- See table on page 5.
- 1.5 mL microcentrifuge tubes
- 4 mil ply plastic bags (or equivalent)

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 39 °C before inserting reactions and setup to run on the FAM channel.

Sample Preparation - Tubers (Skip to Page 2 for testing bacterial culture)

1. Sub-samples of up to 200 tuber cores may be tested with this assay.

2. Using a clean metal coring tool (scoopula, melon baller, etc.) take a core sample from the stolon end of each of the tubers to be tested. For minitubers, use a razor blade to slice off the stolon end section of the tuber. Cores should be cone-shaped and approximately 1 cm to 2 cm in diameter x 2 cm in depth. Sections of mini-tubers should be approximately 1/2 of the mini-tuber. Tuber cores should be free of soil/debris prior to soaking (rinse with distilled water as needed).

NOTE: Coring tools should be sanitized between sub-samples with a 600 ppm bleach solution.

3. Place the cores inside a 4 mil ply plastic bag (right), or equivalent. Add enough distilled water to just cover the surfaces of the cores when the bag is held upright. Seal bag completely.

4. Secure the bag on an orbital shaker and shake overnight at 60 to 90 rpm (enough so that water moves freely around all tuber cores) at room temperature.

5. Gently massage the bag the following day to mix. Pipette 500 µL of the sample extract into a 1.5 mL centrifuge tube. Add 500 µL of AMP1 extraction buffer to this aliquot. Larger aliquots may be taken providing they are diluted 1:1 with AMP1 extraction buffer prior to testing. Incubate the diluted extract for 10 minutes at room temperature, then proceed to the PD1 dilution section **(page 2)** of this user guide.

Click here to access your lot specific QR code

Sample Preparation - Bacterial Culture (Skip if testing tubers)

1. Dispense 500 µL of sterile water into a 1.5 mL microcentrifuge tube (sold separately).

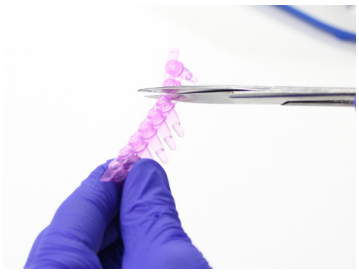
Suspend a loopful/colony in the water from freshly grown culture

If testing a dilution series of culture, make the serial dilutions in sterile water. If you wish to plate the dilutions, do so before proceeding to the next steps.

2. Add an equal volume of AMP1 extraction buffer to the culture suspension/dilution and mix by pipetting. (example: 500 µL suspension: 500 µL AMP1 buffer). Incubate for 10 minutes. Proceed to the PD1 dilution step.

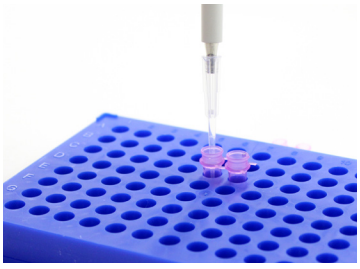
Pellet Diluent (PD1) Dilution (DO NOT SKIP)

1. Remove one colored PD1 filled tube for each sample being tested. Individual tubes may be cut from the strip of tubes using scissors. Be sure to label the caps with your sample identity. **Inspect the tube to ensure all liquid is at the bottom before use.**



2. Transfer 5 µL of sample extract into the tube containing PD1 diluent and **mix well.**

Your samples are now ready to be tested.



NEXT STEP - DETECTION:

page 3. Real-Time Detection via AmpliFire

page 4. End-Point Detection via Amplicon Detection Chamber

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.

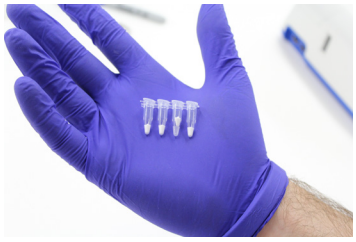
Note: Scanning works best when the barcode is held 3 - 4 inches from the scanner in an area with sufficient ambient light.

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.

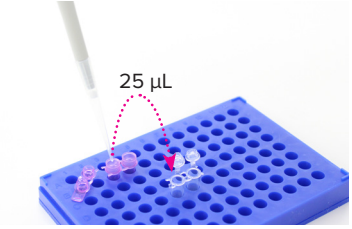
Sample IDs for individual wells are optional. If you prefer to use the default values, click “FINISH”.

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.

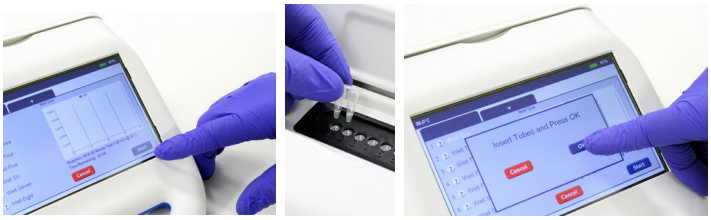
5. Transfer 25 µL from the colored tube containing your sample extract into the reaction pellet (clear tube).



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.

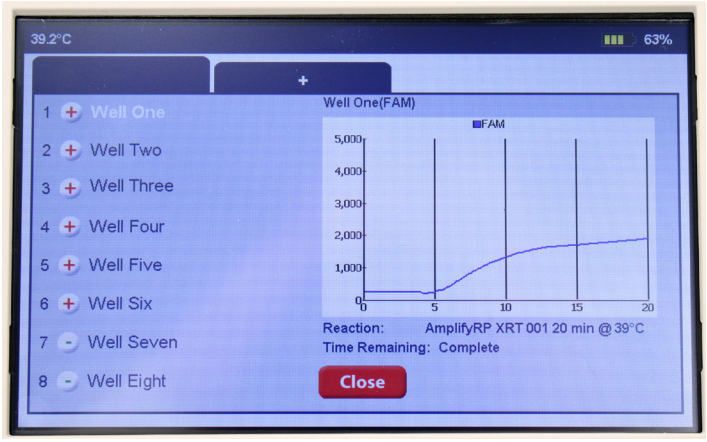
6. Press “Start” on the AmpliFire. Immediately follow the prompts to add your reactions, press “OK”, and put the lid down.



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.

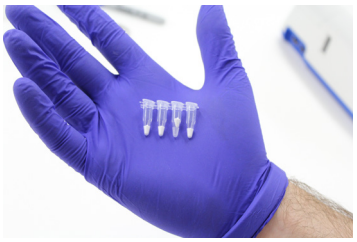
8. After 20 minutes of total run time the instrument will beep, indicating the test is complete. The test results will be visible next to the well designation on the screen, and should be interpreted as follows:

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



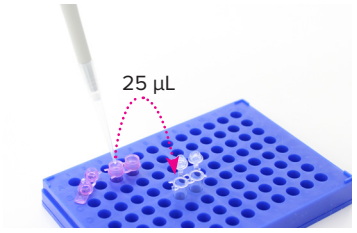
Test Protocol for End-point Detection Using Heat Block/Amplicon Detection Chambers

Amplification



1. Allow heat block to warm to 39 °C before preparing reactions. If using an Agdia-supplied heat block, allow 2 to 3 minutes for this step.

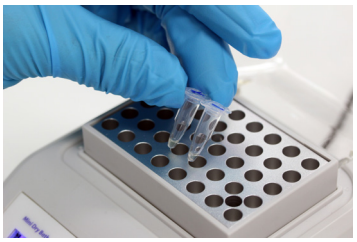
2. Remove the strip of reaction pellets from the desiccated container included in the kit. While securing the strip of pellets in a 200 µL PCR tube rack, cut the number of reaction pellets from the strip that are intended for use. Immediately place remaining reaction pellets back into the desiccated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

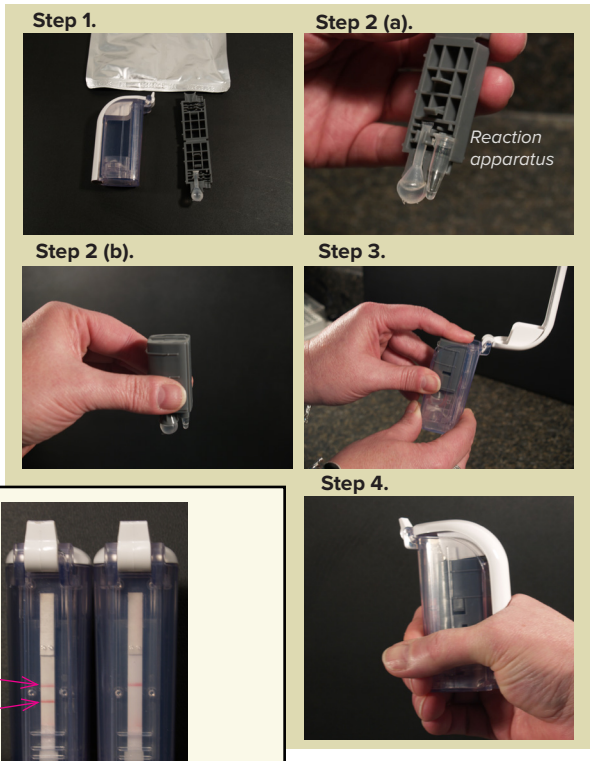
In order to avoid possible contamination of future tests, DO NOT open the reaction pellet.

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.

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