AmplifyRP® XRT for *Dickeya* spp.

Rapid DNA Amplification Test Kit
Product No. XCS 55500

Intended Use:

AmplifyRP XRT for *Dickeya* is a rapid DNA amplification and detection platform designed for testing potato tubers, stem, and bacterial culture for *Dickeya* spp. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify *Dickeya* DNA at a single operating temperature (39 °C).

SPECIFICITY: Detects *Dickeya* spp. Does not cross-react with other species of bacteria.
SENSITIVITY: Approximately 200 CFU/mL
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.)

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.

Contents of Kit:

- Reaction pellets
- 100 µL Pellet Diluent Tubes
- AMP1 extraction buffer
- Sample extraction bags, mesh

Not Included but Required:

- AmpliFire Isothermal Fluorometer AFR 60400 (or equivalent)
- 1.5 mL microcentrifuge tubes
- 4 mil ply plastic bags (or equivalent)
- Pipettes & tips (10 µL, 25 µL, 500 µL)

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) 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 plant tissue and bacterial culture)

1. Sub-samples of up to 25 tuber cores are recommended for testing with this assay. Larger sub-samples may be tested at the end-users discretion, however it is important to note that the limit of detection for this assay is 200 CFU/mL regardless of the sub-sample size. The probability of false negative results may increase with increased sub-sample size due to potential dilution of infected plant material.

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. *Dickeya* bacteria favors anaerobic conditions for growth, therefore; it is important to remove excess air from the bag prior to sealing. Push excess air from bag then seal 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 microcentrifuge 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 - Plant Tissue (Skip if testing tubers or bacterial culture)

1. Select 0.15 g of stem tissue showing signs of infection. It is best to collect stem tissue as close to ground level as possible as the source of the bacteria is most likely from contaminated soil or tubers.

2. Place the tissue inside the provided mesh extraction bag. Add 3.0 mL of AMP1 extraction buffer. This should result in a 1:20 (w/v) extraction ratio of plant tissue. Extract the tissue by thoroughly macerating it with a blunt object such as a pen. Incubate at room temperature for 10 minutes.

3. Proceed to the PD1 dilution step.

Sample Preparation - Bacterial Culture (Skip if testing tubers or plant tissue)

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

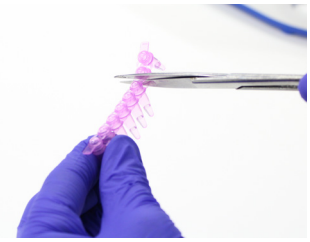
Suspend a loopful/colony in the water from freshly grown culture.

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.

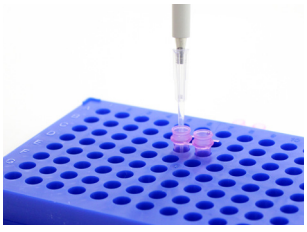
2. Add an equal volume of AMP1 extraction buffer to the culture suspension/dilution and mix by pipetting. (example: 500 µL suspension: 500 µL AMP1). Incubate at room temperature for 10 minutes.

3. 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 10 µL of sample extract into the tube containing PD1 diluent and **mix well.**

Your samples are now ready to be tested. Proceed to the Test Protocol on Page 3.

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.

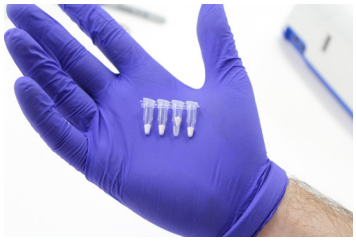
Note: Scanning works best when the barcode is held 3 - 4 inches from the scanner in an area with sufficient ambient light.

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.

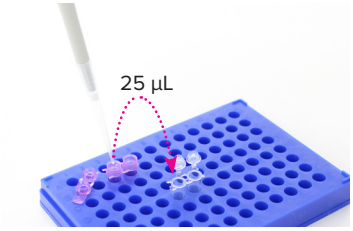
Sample IDs for individual wells are optional. If you prefer to use the default values, click “FINISH”.

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.

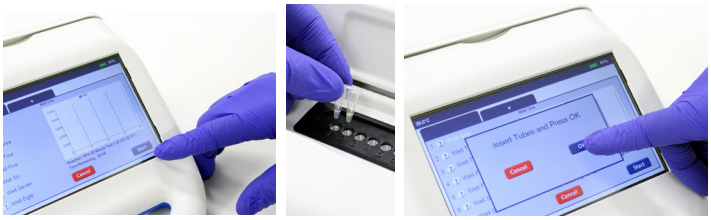
5. Transfer 25 µL from the colored tube containing your sample extract into the reaction pellet (clear tube).



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.

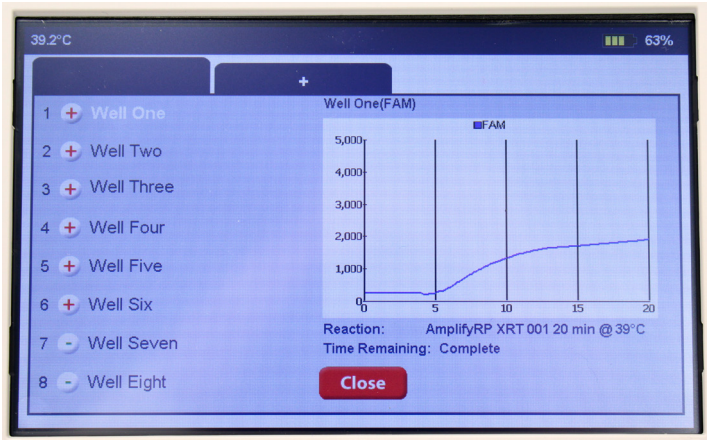
6. Press “Start” on the AmpliFire. Immediately follow the prompts to add your reactions, press “OK”, and put the lid down.



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 *Dickeya* (-) = *Dickeya* not detected (!) = Invalid



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 of the Test Protocol. 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 be 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:

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