

Intended Use:

AmplifyRP XRT for *Foc* TR4 is a rapid DNA amplification and detection platform designed for testing bananas and plantains for *Fusarium oxysporum f. sp. cubense*. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify *Foc* TR4 DNA and an exogenous DNA control at a single operating temperature (42 °C).

SPECIFICITY: Detects only *Foc* TR4. Does not cross-react with *Foc*-R1, R2, R3, and STR4.
SENSITIVITY: Approximately 40 copies of DNA fragments.
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)
- Pipettes (5 µL & 25 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 42 °C before inserting reactions and setup to run on the FAM and CalRed channels.

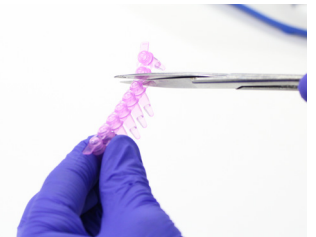
Sample Preparation - Plant Tissue

1. *Foc* TR4 fungus mycelium is most often not evenly distributed throughout an infected plant's vascular system. Therefore Agdia recommends to test pseudostem, stem, rhizome, and root tissue when possible. Collect 0.3 g of tissue from the suspect area.

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

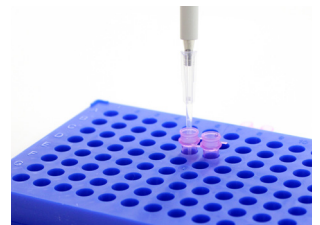


3. Remove one colored PD1 filled tube for each sample being tested. Individual tubes may be cut from the strip of tubes using scissors. Be sure to label the caps with your sample identity. **Inspect the tube to ensure all liquid is at the bottom before use.**



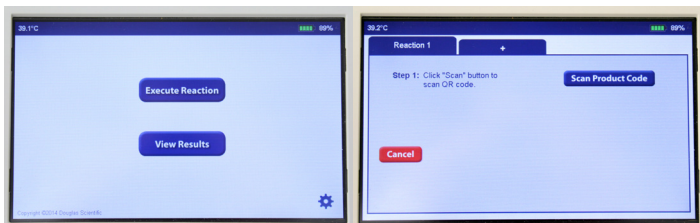
4. Transfer 5 µL of sample extract into the tube containing PD1 diluent and **mix well.**

Your samples are now ready to be tested. Proceed to the Test Protocol on Page 2.



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 located on the foil pouch containing the canisters of reaction pellets. The barcode scanner is located on the left side of the AmpliFire.

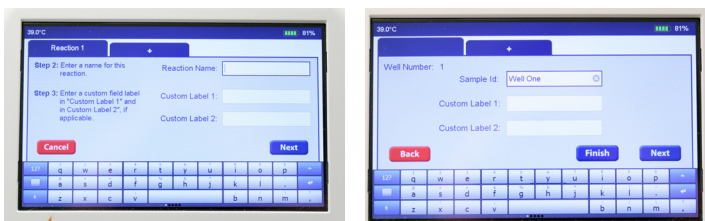


Once the AmpliFire has accepted the scan and displayed run method, click “Next”.

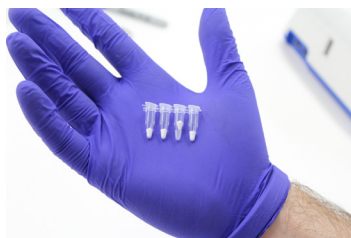


3. Follow on-screen prompts to name your reaction and individual sample IDs.

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

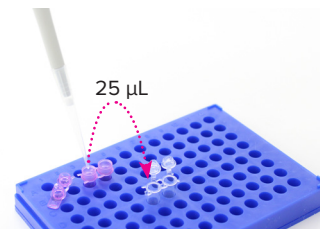


4. Remove a canister of reaction pellets from the white foil pouch labeled with the barcode. Then remove a strip of reaction pellets from the desiccated container. While securing the strip of pellets in a 200 µL PCR tube rack, cut the number of reaction pellets from the strip that are intended for use.



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.

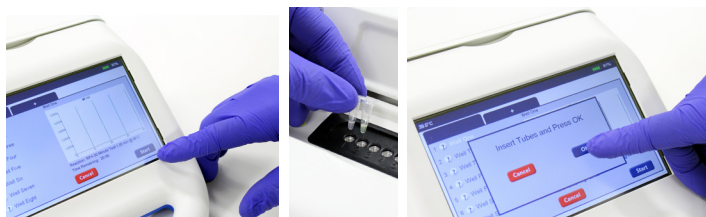
5. Transfer 25 µL from the colored tube containing your sample extract into the reaction pellet (clear tube).



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.



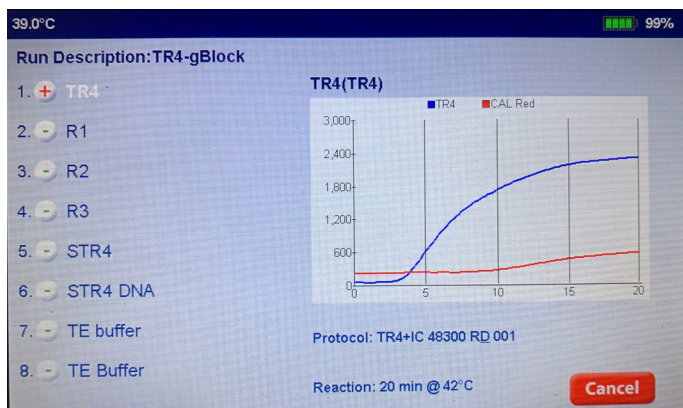
7. After 4 minutes of incubation remove the reaction(s) from the AmpliFire. Quickly mix, spin, and reinsert the reaction(s) into the AmpliFire to continue monitoring results. Take care to ensure the tubes are in their original positions and orientations.

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:

Blue curve = FAM = *Foc* TR4 Red curve = CalRed = Internal control

(+) = Positive for *Foc* TR4 (-) = *Foc* TR4 not detected (!) = Invalid

The internal control is an exogenous reaction and occupies the CalRed channel. An invalid result indicates that the internal control did not amplify as expected (see Limitations on page 3).



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.

Invalid Results: An invalid result indicates that the internal control did not amplify as expected. This phenomenon is usually caused by the sample being too concentrated leading to inhibition of the reaction or from adding too much or too little extract diluted in PD1 to the reaction pellet (See step 5 of the Test Protocol). Always use care to extract the sample at the recommended sample dilution and follow the prescribed dispense volumes in this procedure.

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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AmplifyRP Test Kits employ recombinase polymerase amplification (RPA) technology, developed by TwistDx Limited, U.K. Use of the RPA process and probe technologies are protected by US patents 7,270,981 B2, 7,399,590 B2, 7,435,561 B2, 7,485,428 B2 and foreign equivalents in addition to pending patents.

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