



Intended Use:

AmplifyRP XRT for ToBRFV is a rapid RNA amplification and detection platform designed for testing peppers and tomatoes for *Tomato brown rugose fruit virus*. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify ToBRFV RNA and an endogenous RNA control at a single operating temperature (42 °C).

SPECIFICITY: Detects only ToBRFV. Does not cross-react with other Tobamoviruses.
SENSITIVITY: Approximately 15 fg/μL of RNA transcripts
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:

Kit components should be stored refrigerated (2 - 8 °C), unless specified otherwise below:

***** Reaction pellets should be stored at -10 to -30 °C when not in use. *****

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
- GEB sample extraction bags

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. Symptomatic or asymptomatic tissue may be tested. Agdia recommends sampling leaves or seeds. Collect 0.15 g of leaf tissue or ground seed flour from the suspect area.

2. Place the tissue inside the provided mesh extraction bag containing GEB extraction buffer. Extract the tissue by thoroughly macerating it with a blunt object such as a pen. **Do not put crude extract bags on ice.**



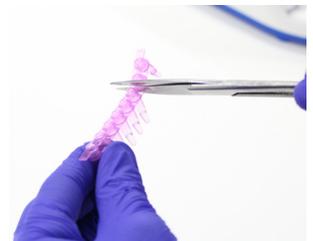
**NOTE:* This test was optimized using a 1:20 tissue to buffer ratio for sample extraction.

ALTERNATIVE EXTRACTION (COMPONENTS NOT INCLUDED)

If preferred, 0.05 g of tissue may alternatively be placed inside a 2.0 mL microcentrifuge tube (sold separately). The tissue can be extracted inside the tube by grinding it with a pestle or golf tee, adding 1.0 mL of GEB extraction buffer, and mixing until the resulting solution is green/brown in color.



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.**

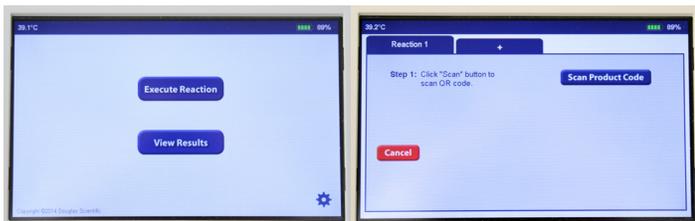


Your samples are now ready to be tested. Proceed to the Test Protocol on Page 2.

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”.

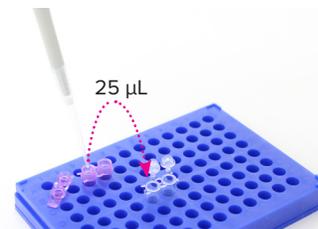


4. Remove a canister of reaction pellets from the white foil pouch labeled with the barcode. Then remove a strip of reaction pellets from the desiccated container. While securing the strip of pellets in a 200 µL PCR tube rack, cut the number of reaction pellets from the strip that are intended for use.



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.

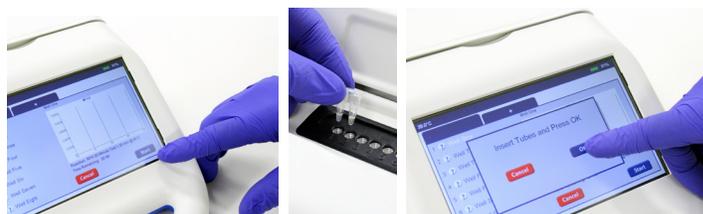
5. Transfer 25 µL from the colored tube containing your sample extract into the reaction pellet (clear tube).



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.



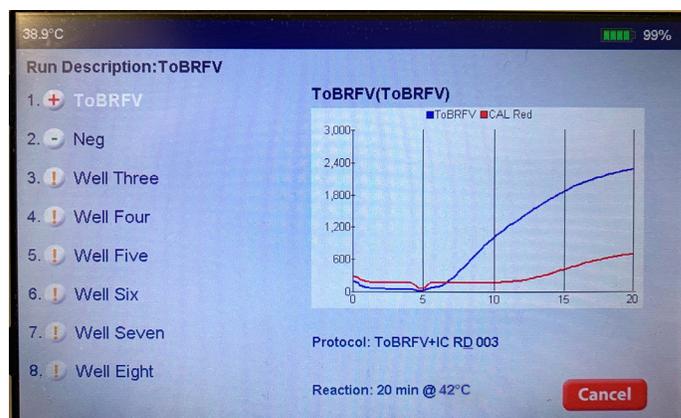
7. After 4 minutes of incubation remove the reaction(s) from the AmpliFire. Quickly mix, spin, and reinsert the reaction(s) into the AmpliFire to continue monitoring results. Take care to ensure the tubes are in their original positions and orientations.

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 = ToBRFV Red curve = CalRed = Internal control

(+) = Positive for ToBRFV (-) = ToBRFV not detected (!) = Invalid

The internal control is an endogenous reaction and occupies the CalRed channel. An invalid result indicates that the internal control did not amplify as expected (see Limitations on page 3). **Note:* The internal control is not applicable when testing pepper tissue using the barcode labeled for pepper.



Limitations

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

Pepper Tissue: Some pepper varieties may not amplify the internal control when using the barcode for tomato, resulting in an “Invalid” test result. If you are testing pepper tissue, please use the barcode labeled for pepper.

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.

Cold buffer: Be sure to warm buffers to room temperature before use. Using cold buffers can cause false positives.

Sample extracts: Do not put crude extract bags on ice. Testing of positive samples on ice has led to diminished signal.

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. If testing pepper tissue and an invalid result occurs, be sure to use the barcode labeled for pepper testing.

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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