AmplifyRP® XRT for CVYV
Rapid RNA Amplification Test Kit
Product No. XCS 14100

Intended Use:
AmplifyRP XRT for CVYV is a rapid RNA amplification and detection platform designed for testing cucurbits for *Cucumber vein yellowing virus*. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify CVYV RNA at a single operating temperature (42 °C).

ASSAY RUN TIME:	20 minutes
SPECIFICITY[1]:	Detects only CVYV. Does not cross-react with other Ipomoviruses.
SENSITIVITY[1]:	Approximately 100 fg/µL of CVYV RNA transcripts
PROBE LABEL:	FAM-labeled target probe
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.

[1]See the validation report for further information.

Contents of Kit:
- Reaction pellets
- 100 µL Pellet Diluent Tubes
- GEB2 sample extraction bags
- Empty 0.2 mL PCR tubes

Not Included but Required:
- AmpliFire Pro Fluorometer AFR 77000 (or equivalent)
- AmplifyRP Heat Block
- Pipettes (5 µL & 25 µL)
- 100 µL barrier pipette tips

Recommended Accessories:
- Mini table-top vortex
- Mini table-top centrifuge

Fluorometers:
This test is designed to run on both AmpliFire Pro and AmpliFire Legacy units. Contact us for information on use with other instruments, such as qPCR instruments or other fluorometers.

The following instructions outline general procedures for both machines. Please reference your equipment instructions for machine specific details.

NOTE:
AmplifyRP is a very sensitive molecular assay. Do not re-use disposable kit components.

It is recommended that disposable gloves be worn when taking samples and performing assay. 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).

Do not get ANY solution into the well block as this could interfere with results.

Heat Block Setup:
Start warming a heat block to 95 °C for use during Sample Preparation.

Questions or Technical Support:

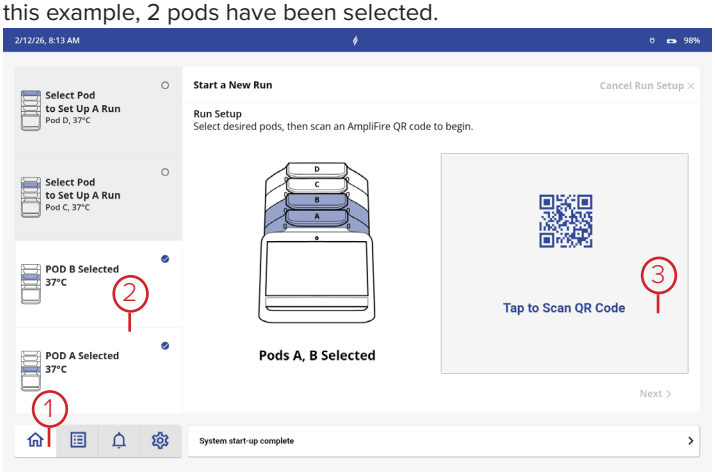
Phone: 800-622-4342 (toll-free) or 574-264-2014
Fax: 574-264-2153
E-mail: info@agdia.com for sales and general product information
techsupport@agdia.com for technical information and troubleshooting
Web: www.agdia.com

Click here to access your lot specific QR code

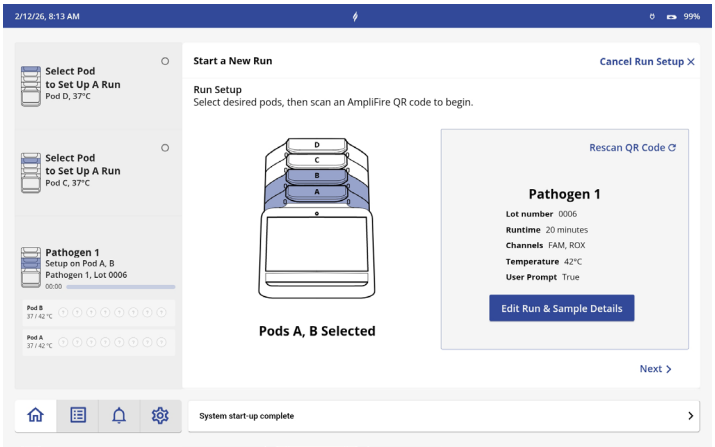
Instrument Setup

AmpliFire® Pro

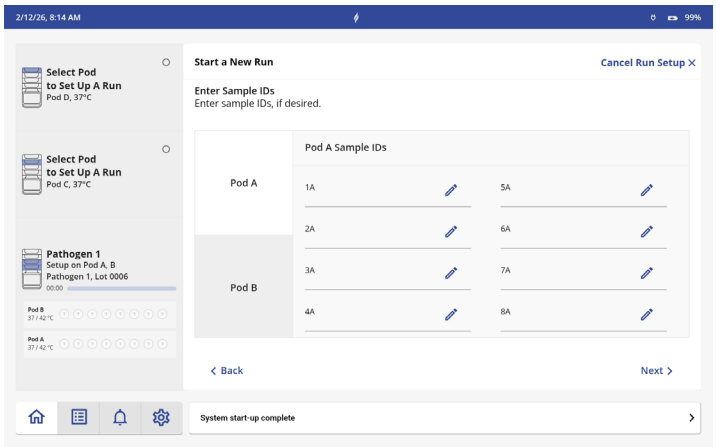
1. From the home page (1) select which pod(s) will be used for the run (2). You can select more than one pod if more than 8 samples are included in the run. Click the 'Tap to Scan QR code' box (3). In this example, 2 pods have been selected.



2. Scan the barcode found by following the hyperlink on page 1. Position the QR code approx. 3 to 6 inches (8 to 15 cm) from Camera Lens, ensuring it is visible in the camera window shown on the screen. Once scan is complete, protocol name will be displayed.

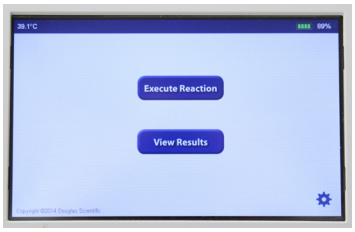


3. If run ID or sample details are to be edited, press "Edit Run & Sample Details". Once finished or if no details are to be edited, press "Next". Selected pods will now stabilize to the target temperature.



AmpliFire® (Legacy)

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".

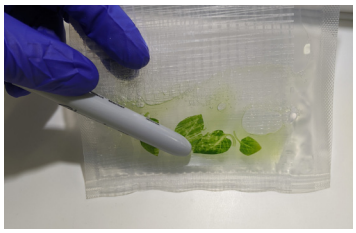
Sample Preparation

Tissue Extraction

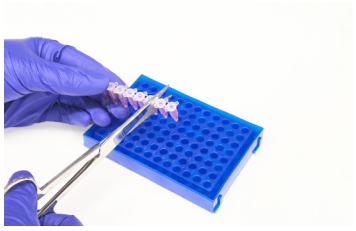
4. Symptomatic or asymptomatic tissue may be tested. Agdia recommends sampling leaves, petioles, or stems. Collect 0.15 g of leaf, petiole, or stem tissue from the suspect area.

5. Place the tissue inside the provided mesh extraction bag containing GEB2 extraction buffer. Extract the tissue by thoroughly macerating it with a blunt object such as a pen.

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

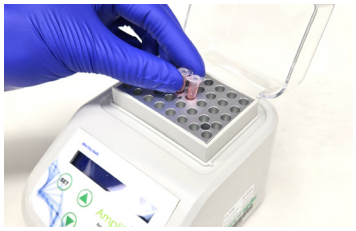


6. Remove one empty PCR tube for each sample being tested. Individual tubes may be cut from the strip of tubes using scissors. Be sure to label the caps with your sample identity.



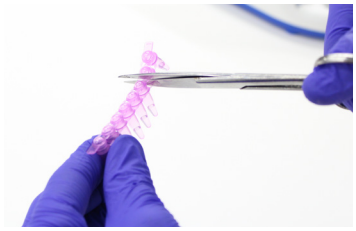
7. Transfer 25 µL of sample extract into the empty tube. **Close lid securely.**

8. Place tube(s) into a prewarmed heat block set to 95 °C and incubate for 5 minutes. **Use caution when placing and removing tubes from the heat block to avoid touching the hot surface and popping open the lids.**



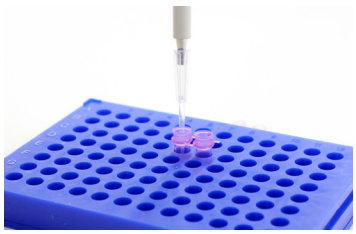
Sample Dilution

9. Remove one colored PD1 filled tube from the “Step 2” foil pouch for each sample being tested. If testing less than 8 samples, individual tubes may be cut from the strip of tubes using scissors. Label the caps with your sample identity. **Inspect the tube to ensure all liquid is at the bottom before use.**



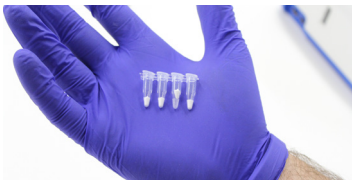
10. Transfer 5 µL of sample extract into the tube containing PD1 diluent and **mix well.**

Your samples are now ready to be tested. Proceed to the Test Protocol on Page 4.



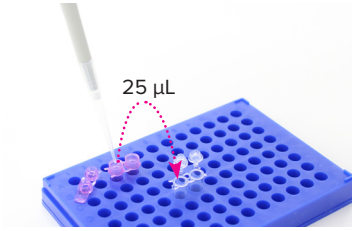
Detection

11. Remove reaction pellets from the “Step 3” foil pouch labeled with the barcode. If using less than 8 reaction pellets, cut the number of pellet tubes from the strip that are intended for use.



Reaction Pellets are light sensitive. Immediately place remaining reaction pellets back into the desiccated tube (8-count) or PCR rack (48 or 96-count) and then place into the foil pouch to protect from light.

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



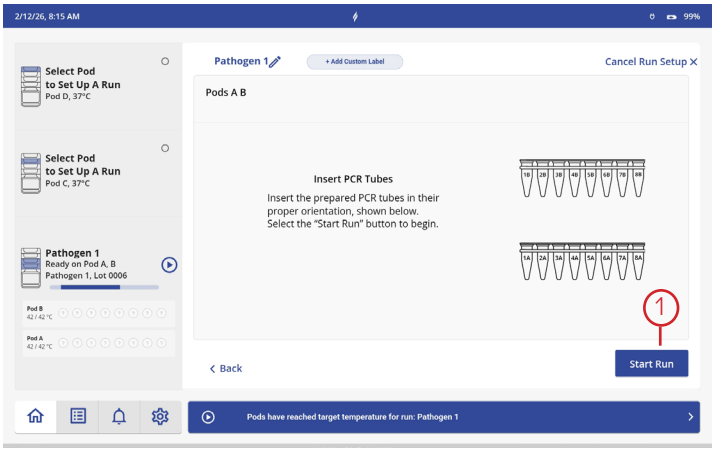
Tightly recap the reaction tube. Mix well and centrifuge. If you cannot vortex the reaction, mix by gently flicking the side of the tube. If you do not have a centrifuge available, you may manually shake the liquid to the bottom of the reaction tube.

IMPORTANT: IMMEDIATELY PROCEED TO THE NEXT STEP ONCE THE REACTION HAS BEEN REHYDRATED. DO NOT RE-OPEN REACTION TUBE AFTER ADDING LIQUID SAMPLE.

Run & Amplification

AmpliFire® Pro

13. Once the pods(s) are temperature-stabilized, the following screen will appear. Insert rehydrated pellet tubes into pod(s) in their correct orientation. Then select “Start run” (1).



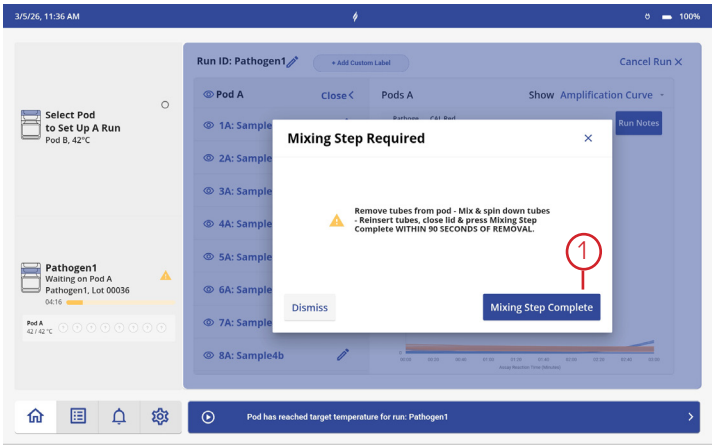
AmpliFire® (Legacy)

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

Mix

AmpliFire® Pro

14. After 4 minutes of incubation, the mix prompt will appear on screen. Remove the reaction(s) from the AmpliFire Pro. Quickly mix, spin, and reinsert the reaction(s) into the AmpliFire Pro. Take care to ensure the tubes are in their original positions and orientations. Once reinserted and the lid has been closed, click “Mixing Step Complete” (1). **Note: Opening the lid outside of the mix window will result in erratic readings and can compromise data.**



AmpliFire® (Legacy)

14. 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.

Results

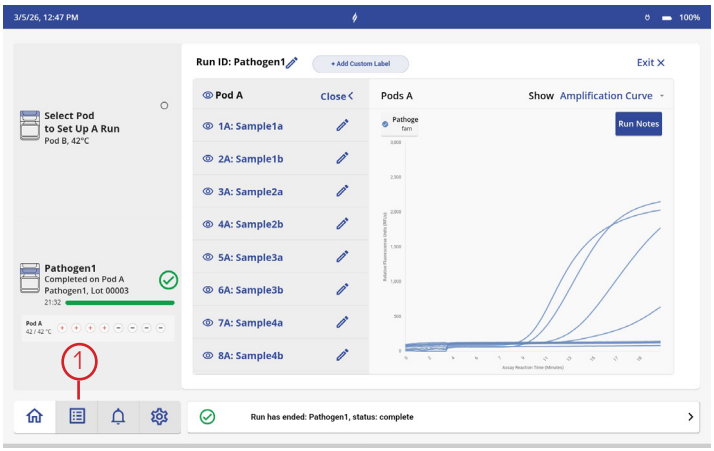
AmpliFire® Pro

15. When the run has completed, the LED lights on the applicable AmpliFire Pro pod(s) will turn green, indicating the test is complete. The test results will be visible below completed pod(s), and should be interpreted as follows:

Blue curve (FAM) = CVYV

(+) = Positive for CVYV, (-) = CVYV not detected, (!) = Invalid

All AmpliFire Pro past results can be viewed by selecting the Run History icon (1).

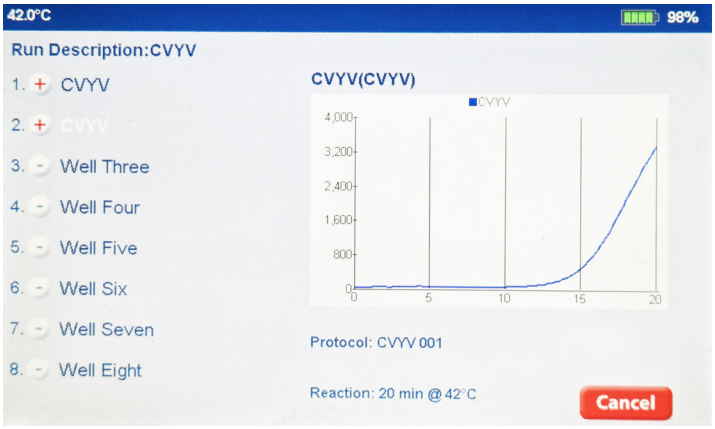


AmpliFire® (Legacy)

15. When the run has completed, AmpliFire will beep to indicate completion. The test results will be visible next to the well designation on the screen, and should be interpreted as follows:

Blue curve (FAM) = CVYV

(+) = Positive for CVYV, (-) = CVYV not detected, (!) = Invalid



Limitations

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

Sensitivity: Skipping the 95 °C incubation step will result in a significant loss in sensitivity.

Purified RNA: If testing purified RNA, the 95 °C incubation step is not needed. 1 µL of RNA can be added directly to the reaction pellet and rehydrated with 24 µL of PD1.

Addition of sample extract to PD1 reaction tube: 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.