AmplifyRP® XRT for CGMMV
Rapid RNA Amplification Test Kit
Product No. XCS 45702

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

SPECIFICITY: Detects only CGMMV. Does not cross-react with other Tobamoviruses.
SENSITIVITY: Approximately 10 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
- GEB2 sample extraction bags
- PCR tubes, 0.2 mL

Not Included but Required:
- AmpliFire Isothermal Fluorometer AFR 60400 (or equivalent)
- AmplifyRP Heat Block ACC 00592
- Seed grinding equipment (seed testing only)
- 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).

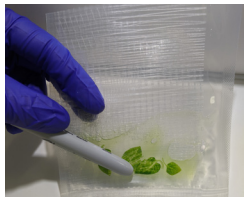
Prior to setting up reactions, turn on the AmpliFire (or an alternative isothermal instrument) so that it is ready to accept reactions. It should be pre-heated to the recommended 42 °C before inserting reactions and setup to run on the FAM and CalRed channels.

Sample Preparation - Plant Tissue (Skip to Page 2 for testing seed)

1. Preheat the heat block to 95 °C.

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

3. 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.

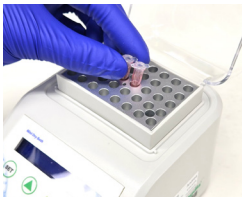


**NOTE*: This test was optimized using a 1:20 tissue to buffer ratio for sample extraction.

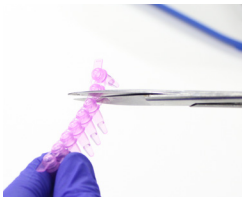
4. Remove one empty PCR tube for each sample being tested. Individual tubes may be cut from the strip of tubes using scissors. Be sure to label the caps with your sample identity.

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

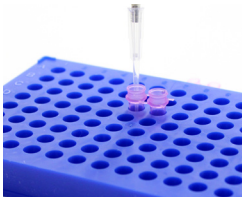
6. Place tube(s) into a prewarmed heat block set to 95 °C and incubate for 5 minutes. **Use caution when placing and removing tubes from the heat block to avoid touching the hot surface and popping open the lids.**



7. 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.**



8. 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 3.

Click here to access your lot specific QR code

Sample Preparation - Seed (Skip if testing plant tissue)

Agdia recomends sampling seed according to International Rules for Seed Testing 7-026: Detection of squash mosaic virus, cucumber green mottle mosaic virus and melon necrotic spot virus in Cucurbitaceae seed (ISHI 2025).

1. Preheat the heat block to 95 °C.

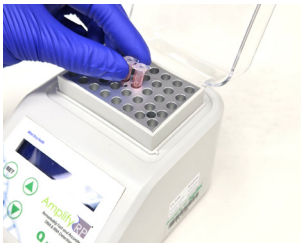
2. Prepare 20 subsamples of 100 seeds each. Dry grind each 100 seed subsample to a fine flour.

3. Weigh 0.5 g of seed flour and combine with 5 mL of GEB2. Mix well to homogenized.

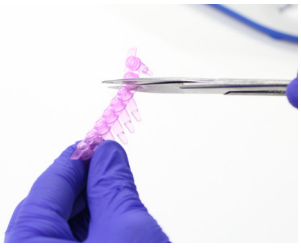
4. Remove one empty PCR tube for each sample being tested. Individual tubes may be cut from the strip of tubes using scissors. Be sure to label the caps with your sample identity.

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

6. 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.**



7. 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.**



8. 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 3.

ISHI *International Rules for Seed Testing 2025 Validated Seed Health Testing Methods 7-026: Detection of squash mosaic virus, cucumber green mottle mosaic virus and melon necrotic spot virus in Cucurbitaceae seed*. 1 Jan. 2025.

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.

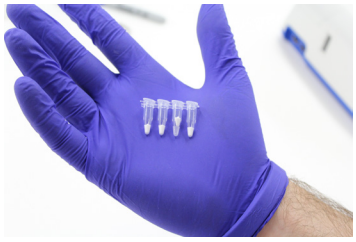
Note: Scanning works best when the barcode is held 3 - 4 inches from the scanner in an area with sufficient ambient light.

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.

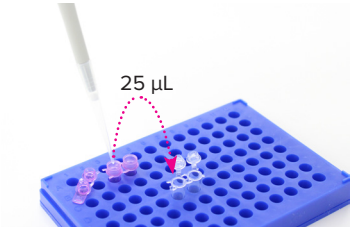
Sample IDs for individual wells are optional. If you prefer to use the default values, click “FINISH”.

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.

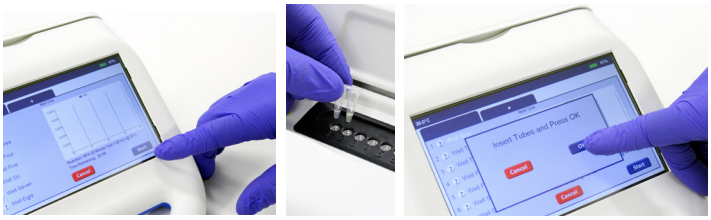
5. Transfer 25 µL from the colored tube containing your sample extract into the reaction pellet (clear tube).



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.



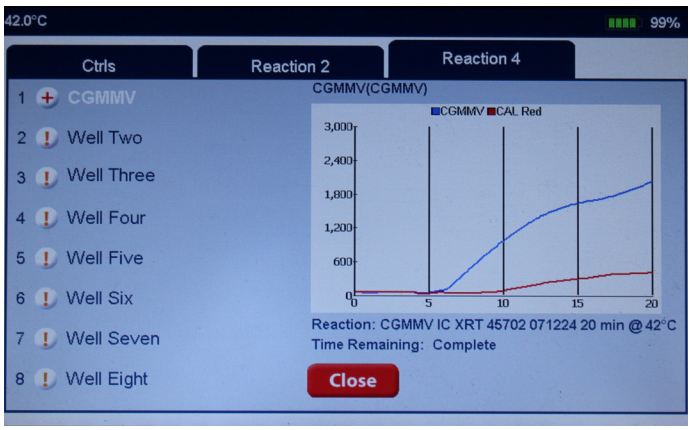
7. After 4 minutes of incubation remove the reaction(s) from the AmpliFire. Quickly mix, spin, and reinsert the reaction(s) into the AmpliFire to continue monitoring results. Take care to ensure the tubes are in their original positions and orientations.

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 = CGMMV Red curve = CalRed = Internal control

(+) = Positive for CGMMV
(-) = CGMMV 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).



Limitations

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

Sensitivity: Skipping the boil step will results in an at least tenfold loss in sensitivity.

Purified RNA: If testing purified RNA no boil is needed. 1 µL of RNA can be added directly to the reaction pellet and rehydrated with 24 µL of PD1.

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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