

AmplifyRP® Acceler8® for Tomato chlorotic dwarf viroid (TCDVd)

Rapid RNA Test Kit

Product No. ACS 65500/0008



Intended Use

AmplifyRP Acceler8 for TCDVd is a rapid RNA amplification and detection platform designed for field-based or laboratory testing of crops such as tomato, petunia, verbena, and calibrachoa for *Tomato chlorotic dwarf viroid*.

This test does not cross-react with CEVd, CLVd, CSVd, HSVd, MPVd, PCFVd, PSTVd, TASVd, or TPMVd.

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 10 to 20 minutes.

Sample Preparation

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 a 10 % bleach solution.

1. Identify plants to be tested. If possible, select fresh leaf material that is showing symptoms (e.g. chlorosis, necrosis, stunting) of TCDVd infection. Collect 300 mg of suspect leaves per sample (be cautious about cross contamination between samples). Material can be selected from multiple leaves of the same plant if desired (Figure 1). Try to select material that includes a portion of the vascular system such as the basal portion of the leaf that includes petiole tissue.
2. Insert the sample between the mesh linings of the GEB3 sample extraction bag. The sample can be extracted by rubbing the outside of the bag with a blunt object such as a pen or marker on a hard surface (Figure 2). Once the sample has been thoroughly homogenized, it is ready to be tested.

**NOTE: This test was optimized using a 1:10 tissue to buffer ratio for sample extraction.*

Amplification

1. Allow heat block to warm to 39 °C before preparing reactions. If using an Agdia-supplied heat block, allow 2 to 3 minutes for this step.
2. Remove the strip of reaction pellets from the desiccated container included in the kit. 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. Immediately place remaining reaction pellets back into the desiccated tube for later use.
3. Dispense 10 µL of PD1 Pellet Diluent into a reaction pellet. Be sure the pellet is visible in the bottom of the tube before opening or dispensing liquid into the tube.
4. Using a 1 µL loop or pipette, immediately transfer 1 µL of sample extract into the rehydrated reaction pellet. Recap the tube and mix by flicking the bottom of the tube 6 to 8 times or by using a laboratory vortex mixer. Then shake or centrifuge the liquid contents to the bottom of the tube.
5. Add reaction to the portable heat block for 15 minutes.
6. Immediately remove reaction from heat block and proceed to detection steps.

Contents of Kit:

- Reaction pellets (8)
- Amplicon detection chambers (8)
- PD1 pellet diluent (1.0 mL)
- GEB3 sample extraction bags (8)
- 1 µL transfer loops (10)

Not Included but Required:

- Portable heat block (ACC 00592)
- 10 µL pipette (ACC 00770/0010)
- 10 µL pipette tips (ACC 00160)
- 200 µL PCR tube rack (ACC 00170)

* A starter pack inclusive of the items not included above can be purchased from Agdia (ACC 00150).

Figure 1. Sample Selection

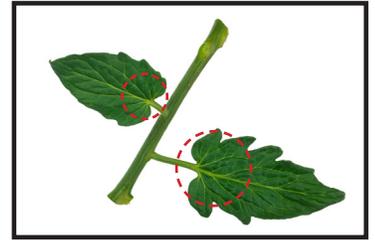


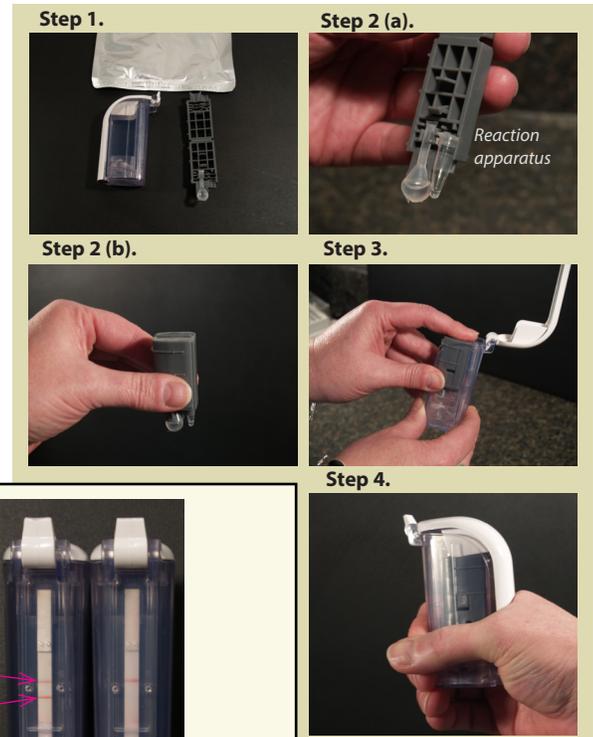
Figure 2. Sample Extraction



Detection

In order to avoid possible contamination of future tests, DO NOT open the reaction pellet.

1. Open the foil pouch containing the Amplicon detection chamber. There are two pieces to the chamber as indicated in the figure to the right.
2. a.) Add the unopened reaction tube to reaction apparatus as illustrated to the right. b.) Once the tube has been added, snap the apparatus shut which will immobilize the reaction tube.
3. Add the reaction apparatus to the detection chamber housing as indicated. **IMPORTANT: The reaction tube should be facing toward the lateral flow strip, contained in the housing, during this step.**
4. Push down on the handle of the detection chamber housing until it snaps shut. Wait 20 minutes before interpreting results. Positive results may be visible in as little as 5 to 10 minutes. Samples that contain lower copy numbers may take up to 20 minutes to produce a positive test line.



Interpret Results

Result	ImmunoStrip Reaction
Positive	Control and Test lines are both visible.
Negative	Control line is visible. Test line not visible.
Invalid	Control line not visible.



Limitations

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

Sample Extraction Buffer: This test must be used with the supplied sample extraction buffer to obtain optimal results.

Addition of sample extract to reaction pellet: It is important to add only the prescribed amount of sample extract to reaction pellets. 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 remain protected from light to prevent bleaching and 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, as this may cause test failure.

Questions or Technical Support:

Phone: 800-622-4342 (toll-free) or 574-264-2014

Fax: 574-264-2153

E-mail: amplifyrp@agdia.com for general questions about this assay or AmplifyRP technology
techsupport@agdia.com for troubleshooting

Web: www.agdia.com