AmplifyRP® Acceler8® for *Grapevine red blotch associated virus* (GRBaV)

Rapid DNA Test Kit

Product No. ACS 57000

Intended Use:

AmplifyRP Acceler8 for GRBaV is a rapid DNA amplification and detection platform designed for field-based or laboratory testing of grape tissue for *Grapevine red blotch associated virus*, the causal agent of grapevine red blotch disease.

Agdia tested the following pathogens and found no cross reactivity with this assay: *Arabis mosaic virus (ArMV), Botrytis cinerea, Grapevine fanleaf virus (GFLV), Grapevine leafroll associated viruses 1-3, or 5 (GLRaV -1, 2, 3, 5), Grapevine fleck virus (GFkV), Tomato ringspot virus (ToRSV), Tobacco ringspot virus (TRSV), or Xylella fastidiosa (Xf).*

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.

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. Symptoms of GRBaV in an infected plant commonly include reddish blotching of leaves and / or reddening of secondary veins (Figure 1). For best results, Agdia recommends testing the basal portion , including petiole, of suspect leaf samples.

2. Cut 0.3 g leaf or petiole in weight. Make certain you clean cutting instruments between samples with a 10 % bleach solution to avoid contamination.

3. Insert the sample between the mesh linings of the buffer filled extraction bag. Each bag contains 3 mL of extraction buffer. The sample can be extracted by rubbing the outside of the bag with a blunt object such as 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 20 minutes (Figure 3).

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)
- GEB2 sample extraction bags, mesh (8)
- 1µL transfer loops (10)

Not Included but Required:

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

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

Figure 1. GRBaV Infected Leaf

Figure 2. Sample Extraction

Figure 3. Add Reaction To Heat Block

Detection

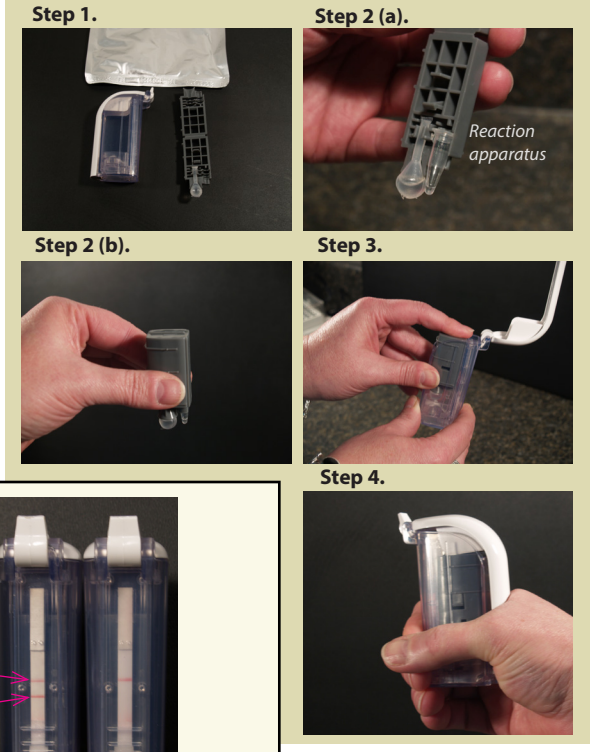
In order to avoid possible contamination of future tests, DO NOT open the reaction pellet.

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:

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