AmplifyRP® Acceler8® for FOV4

Rapid DNA Amplification Test Kit

Product No. ACS 19700

Intended Use:

AmplifyRP Acceler8 for FOV4 is a rapid DNA amplification and detection platform designed for field-based or laboratory testing of cotton crops for *Fusarium oxysporum* f.sp. *vasinfectum*, Race 4. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify FOV4 DNA at a single operating temperature (39 °C).

SPECIFICITY: Detects FOV4 and an Egyptian race 3 isolate, FOV1857. Does not cross-react with other cotton pathogens.

SENSITIVITY: Approximately 400 copies per reaction.

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.

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).

Contents of Kit:

- Reaction pellets
- Amplicon detection chambers
- Pellet Diluent
- GEB3 sample extraction bags
- 1 µL transfer loops

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).

Sample Preparation

1. Plants indicative of FOV4 will show signs of wilt or pre-mature death of the plant. Identify suspect plant and place entire plant with roots, stem and leaf intact into a zip lock bag. Before testing, rinse the roots and stems to remove all traces of soil from the sample.
2. Cut the stem vertically to detect signs of vascular staining in the sample. If vascular staining is present, cut about ½ ” to 1 ” in length of the stem, near the root as the preferred sampling method. 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 a pen or marker on a hard surface (Figure 2). Once the sample has been thoroughly homogenized, it is ready to be tested. (NOTE: There may be stem tissue left in the mesh bag that is not thoroughly ground.)

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 Pellet Diluent 1 (PD1) 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 (Figure 3).
6. Immediately remove reaction from heat block and proceed to detection steps.

Figure 1. Vascular Staining

Photo courtesy of Mike Davis, UC Davis

Figure 2. Sample Extraction

Figure 3. Add Reaction To Heat Block

Detection

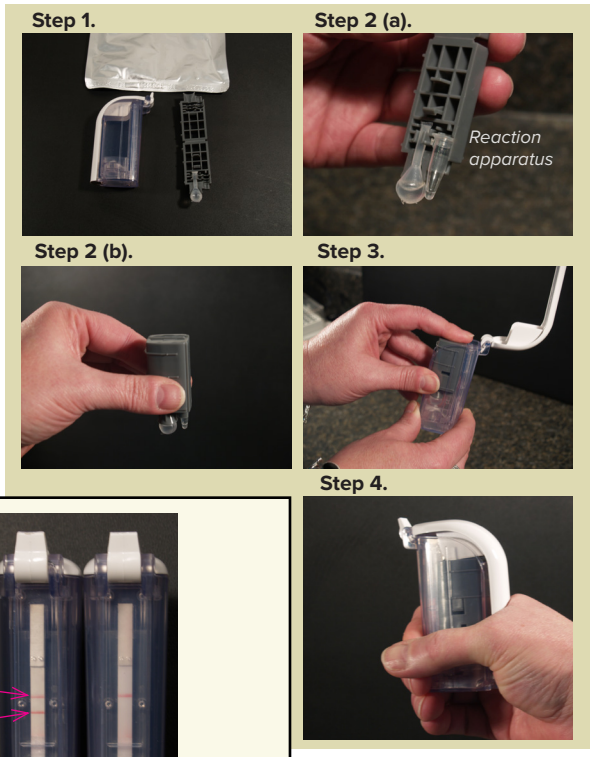
In order to avoid possible contamination of future tests, DO NOT open the reaction pellet.

1. Open the foil pouch containing the Amplicon Detection Chamber (ADC). 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.

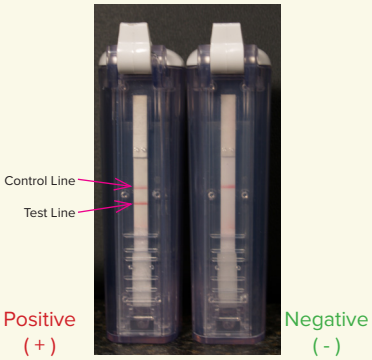
3. Add the reaction apparatus to the detection chamber housing as indicated. **IMPORTANT: The reaction tube should be facing toward the lateral flow strip, contained in the housing, during this step.**

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	Lateral Flow Strip 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, even for short periods of time, 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: info@agdia.com for sales and general product information
techsupport@agdia.com for technical information and troubleshooting

Web: www.agdia.com