AmplifyRP® Acceler8® - Discovery

Rapid DNA / RNA Amplification Test Kit

Product No. ACS 98800

Intended Use:

AmplifyRP Discovery Kits include lyophilized reaction pellets that contain all the reagents necessary to amplify DNA or RNA (reverse transcriptase required) at a single operating temperature (39 - 42 °C). The end user needs only to supply suitable primers and probes for their target of interest. If planning to amplify RNA, the addition of reverse transcriptase to each reaction will also be necessary.

NOTE: This user guide is a basic instructional document that assumes suitable primers and probes have been designed for use in AmplifyRP Acceler8 technology. For detailed recommendations on assay design, including sample extraction suggestions, please view our Assay Design Help Book which can be downloaded from our website, www.agdia.com.

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.

Contents of Kit:

- Reaction pellets
- Rehydration buffer
- Magnesium acetate, 280 mM

Not Included but Required:

- Amplicon Detection Chambers (ADC 98800/0001)
- Portable heat block (ACC 00592)
- Suitable AmplifyRP Primers & Probe
- Sample extraction materials
- Dispensing equipment
- Reverse transcriptase (RNA only)

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).

Sample Preparation

AmplifyRP technology may be used on crude extracts and does not require special preparation of nucleic acids. Your method of extraction will depend on the type of pathogen you are targeting and the host tissue. Agdia has found that many common extraction buffers are suitable for use with AmplifyRP technology. In many cases extracting plant tissue with a mesh extraction bag or a mortar and pestle is sufficient. See Agdia's AmplifyRP Discovery Kit Assay Design Help Book for more information regarding sample extraction.

Test Protocol

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. For each sample, prepare a rehydration solution as follows:

• Rehydration buffer	5.90 µL
• Primer A (10 µM)	0.42 µL*
• Primer B (10 µM)	0.42 µL*
• Acceler8 probe (10 µM)	0.12 µL*
• Magnesium acetate, 280 mM	0.50 µL
• Reverse transcriptase (see note below)	
• dH20	fill to 9.00 µL

*The volumes for primers, probe, and water are suggested starting points for test development. They may change once the test is fully optimized.

WARNING

Reaction pellets are activated once magnesium acetate has been added, even at room temperature. It is recommended to proceed to the incubation step quickly once the pellet has been rehydrated.

NOTE: Reverse transcriptase (RT) should be added to the rehydration solution if testing RNA. The recommended concentration for RT should be 20 units per reaction for the Acceler8 format.

4. Add 1.00 µL of sample to the side of the reaction pellet. Transfer 9.00 µL of rehydration solution to the reaction pellet, for each sample.

5. Tightly recap the reaction tubes. Vortex and centrifuge to start the reaction. 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.

6. Transfer the reaction tube(s) to a portable heat block and incubate for 20 minutes at 39 °C.

7. Immediately remove reaction from heat block and proceed to detection steps.

Detection

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

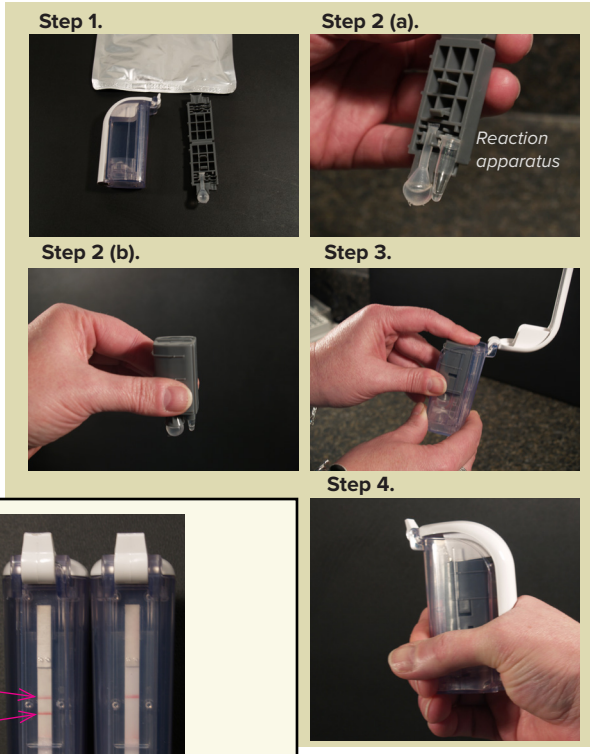
Amplicon Detection Chambers sold separately (Item No. ADC 98800/0001)

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.

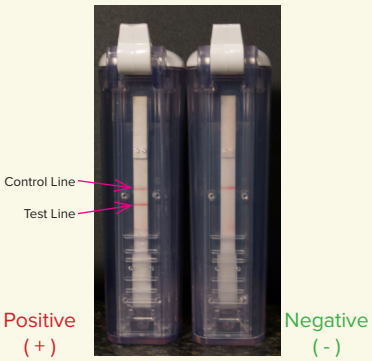
3. Add the reaction apparatus to the detection chamber housing as indicated. **IMPORTANT: The reaction tube should be facing toward the lateral flow strip, contained in the housing, during this step.**

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.

Reaction Volume: Care should be taken to ensure the volume used to rehydrate the reaction is within +/- 10% of the prescribed 9 µL mentioned in step 3 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 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.

Permitted Use of Product: The end-user of this product acknowledges and agrees that the materials and information provided in AmplifyRP Discovery Kits are for RESEARCH purposes only in the following Field of Use:

- Detection of plant pathogens that cause disease in crops
- Detection of non-native genes in crops
- Detection of native genes in crops, except *Cannabis sativa*

End users are restricted from use of AmplifyRP Discovery kits outside the aforementioned Field of Use. AmplifyRP Discovery kits are not to be used for commercial purposes or to provide services to any third party.

Questions or Technical Support:

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