



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 XRT 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.

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. Prior to setting up reactions, set up your fluorometer so that it is ready to accept reactions.

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 14.75 µL
- Primer A (10 µM) 1.05 µL*
- Primer B (10 µM) 1.05 µL*
- XRT probe (10 µM) 0.3 µL*
- Reverse transcriptase
(see note below)
- dH2O fill to 22.75 µL

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

NOTE: Reverse transcriptase (RT) should be added to the rehydration solution if testing RNA. The recommended concentration for RT should be 50 units per reaction for the XRT format.

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.

Contents of Kit:

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

Not Included but Required:

- AmpliFire® Isothermal Fluorometer [AFR 60400](#) (or equivalent)
- Suitable AmplifyRP XRT Primers
- Suitable AmplifyRP XRT Probe
- Sample extraction materials
- Dispensing equipment
- Reverse transcriptase (RNA only)

4. Add 1.25 µL 280 mM magnesium acetate to the cap of each reaction pellet. Add 1.00 µL of sample to the side of the reaction pellet. Transfer 22.75 µL 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. Immediately place into fluorometer and begin monitoring reaction(s).

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

8. Onset of amplification (if positive) will typically occur between 6 and 10 minutes for higher titer samples and 10 and 20 minutes for lower titer samples. This greatly depends on your primer and probe design and how well they have been optimized. Reactions should not be allowed to incubate for more than 30 minutes.

This is a generic barcode to run at 39 °C for 20 minutes and display FAM, HEX, and CalRed channels on the AmpliFire.

If you would like to run at either a different temperature or length of time, please contact techsupport@agdia.com



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 22.75 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 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.

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:

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

AmplifyRP Test Kits employ recombinase polymerase amplification (RPA) technology, developed by TwistDx Limited, U.K. Use of the RPA process and probe technologies are protected by US patents 7,270,981 B2, 7,399,590 B2, 7,435,561 B2, 7,485,428 B2 and foreign equivalents in addition to pending patents.

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