

CRD™ Rapid Botrytis Test Kit

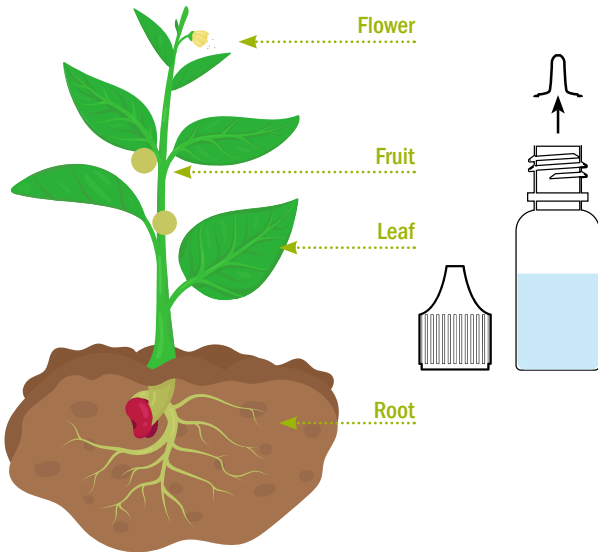
Instructions for Testing of Plant Material

Please read in full
before use



SAMPLE PREPARATION

1 Remove bottle cap and nozzle from buffer bottle. Cut up to 10 plant material samples, you can use the bottle cap as if a cookie cutter. If the plant is exhibiting disease, select samples which fall across the disease and healthy zones. Avoid dried or dead plant material. For roots, clean and make free of soil. For root, fruit and stem material break up and section into small pieces before adding to the bottle.



! For asymptomatic plant material 6+ hrs of sample incubation in the buffer can stimulate germination and be used as an Early Detection Test. The test detects Botrytis mycelium rather than the spore.

Too much plant and particulate material can cause the test membrane to become blocked and void the test. Formation of a control line (reading your test) will indicate if the test has run correctly.

2 Place the plant samples into the buffer bottle and add three carbon steel balls. Insert the nozzle and secure the lid. Shake the bottle for 15 seconds to break up the tissue. Stem, fruit, and root material may require longer (30 seconds) of vigorous shaking.

3 If you wish to test immediately proceed to "Running Your Test". If you are performing an Early Detection Test add a germination pad to the sample bottle and leave it in a dark location for 6-24 hrs before performing the test. See Reading Your Test #2 for more info.

RUNNING YOUR TEST

1 Remove the buffer bottle cap. Invert the bottle over an empty test well and gently squeeze the bottle to dispense 5-10 drops of the solution into the test well.

2 Remove one Botrytis test strip from the resealable container. Ensure the container is immediately resealed following the removal of a test strip.

3 Before running a test ensure the plant material and buffer are at room temperature.

4 While holding the red handle of the Botrytis test strip place the other end into the sample cup. The liquid in the sample cup should not exceed the top of the blue band on the sample strip. Leave the Botrytis test strip to run upright in the sample for exactly 10 minutes.

5 At 10 minutes a control line should be clearly visible. Depending on your sample a test line may also be visible.

Remove the test strip from the sample cup. Read immediately using either a score card or a CRD Cube Reader*. Ensure you read your test at 10 minutes and if again, no later than 20 minutes.

The test can be used to provide a semi-quantitative indication of the presence of Botrytis cinerea. The test line will change over time. Do not read after 20 minutes.

READING YOUR TEST

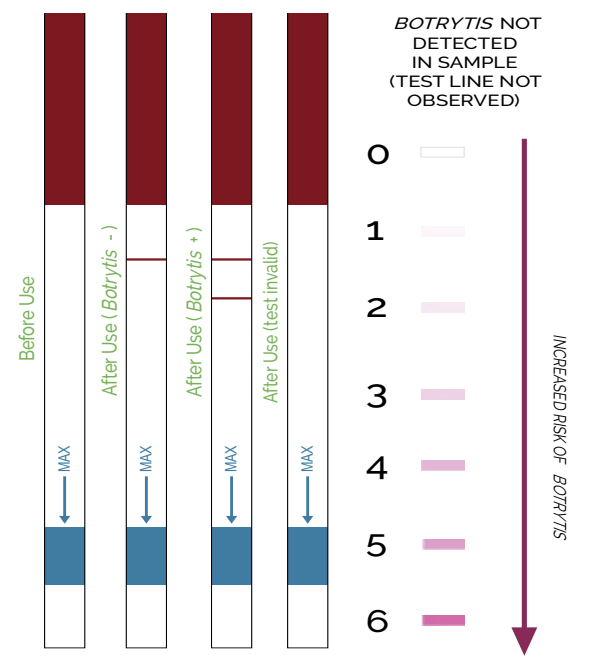
1 Read your test strip visually using the score card.

The control line will indicate if the test has worked. If the control line does not appear re-test with a new test strip and review the troubleshooting section.

The presence of both the control and test lines indicates a positive result, regardless of test line intensity.

If only the control line is visible this indicates a negative result.

LATERAL FLOW TEST LINE SCORE CARD



2 **Early Detection Test**

CRD™ Botrytis has the ability to detect spores and low level or dormant levels of botrytis. This can be done by letting the sample germinate over 6-24 hrs and then testing for new mycelium.

If an Early Detection Test is desired, store the sample in its buffer bottle with the lid securely fastened. The bottle should be kept in dark location for 6-24 hrs. After 6+ hrs follow "Running Your Test" instructions.

User Guide

KIT INFORMATION

Test Kit Intended Use

The CRD™ Rapid Botrytis Test Kit is a rapid lateral flow assay (LFA) test for the presumptive detection of the fungus *botrytis cinerea*. It is designed for field-based or laboratory testing of plant material. This kit includes buffer solution, test strip and all other necessary components to perform a complete test.

Kit Storage and Shelf Life

Test kits and all test kit components should be stored between 35-70 F° (2-20° C). Test kits should be stored out of direct sunlight and not in a vehicle. Test kits should be stored in a dry environment. All test kit components should remain sealed when not in use to prevent moisture degradation, which may affect test results.

Test kits have a shelf life of 12 months from the date of manufacture if stored in proper conditions.

Contents of Test Kit

- Everything included to run full test

- 25 Botrytis test strips (LFA0010)
- 25 Buffer dropper bottles with solution (LFB0020)
- 25 Test wells (LFW0030)
- 25 Buffer solution pads (LFP0040)
- 75 Carbon steel balls
- Instructions / User Guide
- Packing insert and test well holder

*Test kit does not include CRD™ Cube Reader for quantitative reading of test strips

TEST TECHNICAL DETAILS

This test does not discriminate viable and non-viable botrytis infection states. This test should not be used to assess the efficacy of a control treatment. For example, a control treatment may be applied and kill botrytis. However the botrytis antigen will likely remain and still be detected by the test.

The test is a preliminary screen and it is recommended that results be confirmed by an alternate method. The test result will depend on the sample assessed, the sample size and the sampling process. A negative result does not preclude the presence of botrytis. As the test may well be used for field & protected crop testing activities protect the test during storage by avoiding hot or cold temperature extremes.

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

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

Addition of sample to buffer solution - It is important to add only the prescribed amount of sample to the buffer solution. Adding too much may impair sample preparation.

Storage - Test results may be weak or test may fail if the storage instructions are not followed properly.

Validated Plant Materials - This test has not been validated for all plant materials and species. Test performance will vary based on material and species.

Safety

Test strips and sample extraction buffer are non-hazardous.

Cross-reactivity

The test and buffer is not known to have reactivity to other plant pathogens or fungi.

TROUBLESHOOTING

The control line did not develop

If no control line is present, results should be considered invalid, and the test should be repeated.

Too much plant and particulate material can cause the test membrane to become blocked and void the test. Formation of a control line will indicate if the test has run correctly.

The test runs very slow or not at all

This can be caused by using too much tissue for extraction. Repeat the test using less tissue. If this is not the case, make sure the test components are within their expiration date.

The test has a green or pigmented test line

During vigorous shaking, plant material will fragment to release Botrytis (if present) into solution. At this time, colored plant pigments may also be released, which can cause the test strip to have a background color. However, the red test line (if Botrytis present) and the control line should be clearly visible. If this occurs, read the test as normal. However, if an excess of green plant sap / chlorophyll is observed to block reading or form a green line, the test is void and will need to be carried out again with less sample and / or less vigorous shaking.

The test and/or control line is hard to read

Make sure the test is within its expiration date. If kit contents were left open too long, the strips could have absorbed moisture, which can affect test results. Be sure to always keep test strips in their vial and tightly sealed between uses.

A faint indication line still indicates a positive test for the target pathogen.

For further questions please visit our website FAQ at www.cubedlabs.com or email us at support@cubedlabs.com