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# *Botrytis cinerea* PCR Detection Kit — Product Insert

**Product # 29400**

## Pathogen Information

*Botrytis cinerea* is a ubiquitous fungal pathogen, often present as a latent infection, which can in some cases later develops into damaging symptomatic infections in a wide range of plant species including economically important crops such as vegetables, ornamentals, bulbs and fruits. Consequently, an apparently healthy plant can deteriorate suddenly due to the development of latent or quiescent infection into visible disease. Sensitive and reliable methods to monitor the fungus in plant tissue are essential to enable the study of the factors affecting the progression from latent to aggressive disease, and to allow for early detection and diagnosis of the disease.

## Principle of the Test

Norgen's *Botrytis cinerea* PCR Detection Kit constituents a ready-to-use system for the isolation and detection of *Botrytis cinerea* using end-point PCR. The kit first allows for the isolation of *B. cinerea* DNA from the fungi samples using spin-column chromatography. The *B. cinerea* DNA is isolated free from inhibitors, and can then be used as the template in a PCR reaction for *B. cinerea* detection using the provided *Botrytis cinerea* Master Mix. The *B. cinerea* Mastermix contains reagents and enzymes for the specific amplification of a 381 bp region of the genome. In addition, Norgen's *B. cinerea* PCR Detection Kit contains a second Mastermix, the PCR Control Master Mix, which can be used to identify possible PCR inhibition and/or inadequate isolation via a separate PCR reaction with the use of the *PCR control (PCRC)* or *Isolation Control (IsoC),* respectively. This kit is designed to allow for the testing of 24 samples.

## Kit Components:

| Component | Contents |
|---|---|
| Lysis Solution | 15 mL |
| Binding Buffer | 9 mL |
| Wash Solution | 9 mL |
| Elution Buffer | 3 mL |
| Bead Tube | 24 |
| Spin Columns inserted in Collection Tubes | 24 |
| Elution tubes (1.7 mL) | 24 |
| **BC 2x Detection PCR Master Mix** | 0.35 mL |
| **Control 2x PCR Master Mix** | 0.35 mL |
| **Isolation Control (IsoC)[*a]** | 0.3 mL |
| **BC Positive Control (PosC)[*b]** | 0.1 mL |
| *Nuclease Free-Water* | 1.25 mL |
| Norgen's DNA Marker | 0.1 mL |
| Product Insert | 1 |

[a] *The isolation control is a cloned PCR product.*
[b] *The positive control is B. cinerea genomic DNA*

## Customer-Supplied Reagents and Equipment

- Disposable powder-free gloves
- Benchtop microcentrifuge
- 1.5 mL microcentrifuge tubes
- 65℃ water bath or heating block
- 96 – 100% ethanol
- 70% ethanol
- RNase A (optional)
- Lyticase (optional)

**Storage Conditions and Product Stability**
All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers.

The BC 2x PCR Master Mix, Control 2x PCR Master Mix, BC Positive Control (*PosC*) and the Isolation Control (*IsoC*) should be kept tightly sealed and stored at -20°C for up to 1 year without showing any reduction in performance. Repeated thawing and freezing (> 2 x) should be avoided, as this may reduce the sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots.

**General Precautions**
The user should exercise the following precautions when using the kit:

- Use sterile pipette tips with filters.
- Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Work quickly on ice.

**Quality Control**
In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's *Botrytis cinerea* PCR Detection Kit, including the BC 2x PCR Master Mix, Control 2x PCR Master Mix, Isolation Control and BC Positive Control are tested against predetermined specifications to ensure consistent product quality.

**Product Use Limitations**
Norgen's *Botrytis cinerea* PCR Detection Kit is designed for research purposes only.

**Product Warranty and Satisfaction Guarantee**
NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

**Precautions and Disclaimers**
This kit is designed for research purposes only. It is not intended for human or diagnostic use.

The **Lysis Solution** contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

**Safety Information**
Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at www.norgenbiotek.com.

**CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.**

## Protocol

- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- Prepare a working concentration of the **Wash Solution** by adding 21 mL of 96 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution**. This will give a final volume of 30 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Lysate can be prepared from either fungi growing on plates, plant tissue or fruit. Please ensure that you follow the proper procedure for lysate preparation in **Step 1a.**
- For the isolation of genomic DNA from fungi growing on plates, **Collection Solution** must be prepared. **Collection Solution** consists of 0.9% (w/v) NaCl prepared with distilled water.
- Preheat a water bath or heating block to 65°C.
- **Optional:** The DNA yield from some fungal species may be increased by performing an optional Lyticase lysis step. If desired, prepare a Lyticase stock solution according to the supplier's instruction. It is recommended that the stock solution have a minimum concentration of 4 units per µL. Aliquot and store the unused portions at -20°C until needed.
- **Isolation Control (*IsoC*)**
    - An Isolation Control (*IsoC*) is supplied. This allows the user to control the DNA isolation procedure. For this assay, add the Isolation Control *(IsoC)* to the lysate during the isolation procedure
    - The Isolation Control *(IsoC)* must not be added to the sample material directly.
    - Do not freeze and thaw the Isolation Control *(IsoC)* more than 2 times.
    - The Isolation Control *(IsoC)* must be kept on ice at all times during the isolation procedure.
- The PCR components of the *Botrytis cinerea* PCR Detection Kit should remain at -20°C until DNA is extracted and ready for PCR amplification.
- It is important to work quickly during this procedure.

**1. Lysate Preparation (Fungi Growing on Plates or Culture)**

a. **Fungi Growing on Plates:** Add approximately 5 mL (volume can be adjusted based on density of fungal growth) of **Collection Solution** (see notes before use) to the plate and gently collect fungal spores and mycelium with an inoculation loop or autoclaved pipette tip, ensuring not to collect any agar debris. Transfer up to 1 mL of washed spores and wet mycelium to a microcentrifuge tube (provided by user).

**Fungi in Culture:** For fungi growing in a culture, transfer 50 mg of fungi (wet weight) to a microcentrifuge tube.

b. Centrifuge at 14,000 x g (~14,000 RPM) for 1 minute to pellet the cells. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.

c. Add 500 µL of **Lysis Solution** to the cell pellet. Resuspend the cells by gentle vortexing.

**(Optional RNase A treatment)** If RNA-free genomic DNA is required, add the equivalent of 10 KUnitz of RNase A (not to exceed 20 µL) to the cell suspension.

d. Transfer the mixture to a provided Bead Tube and secure the tube horizontally on a flatbed vortex pad with tape, or in any commercially available bead beater equipment (e.g.Scientific Industries' Disruptor GenieTM).

e. Vortex for 5 minutes at maximum speed or optimize the condition for any commercially available bead beater equipment

**Note:** Foaming during the homogenization is common. This foaming is due to detergents present in the Lysis Buffer and will not affect the protocol.

f. Incubate the **Bead Tube** with lysate at 65℃ for 10 minutes. Occasionally mix the lysate 2 or 3 times during incubation by inverting the tube.
g. Briefly spin the tube to remove liquid from the cap, and transfer all of the lysate, including cell debris, to a DNase-free microcentrifuge tube (provided by the user) by pipetting.
h. Centrifuge the tube for 2 minutes at **14000 × g (~14,000 RPM)**.
i. Carefully transfer clean supernatant to a new DNase-free microcentrifuge tube (provided by the user) without disturbing the pellet. Note the volume.
j. Add an equal volume of 96%-100% ethanol (provided by the user) to the lysate collected above (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix.
k. Add 300 µL of **Binding Solution** and briefly vortex to mix.

**2. Binding Nucleic Acids to Column**

a. Obtain a spin column assembled with its collection tube.
b. Add 10 µL of **Isolation Control (*IsoC*)** to the lysate mixture.
c. Apply up to 650 µL of the lysate with ethanol onto the column and centrifuge for 1 minute at **6,000 x g (~8,000 RPM)**. Discard the flowthrough and reassemble the spin column with the collection tube.

   **Note:** Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute.

d. Repeat step **2c** with remained lysate.

**3. Column Wash**

a. Apply 500 µL of **Wash Solution** to the column and centrifuge for 1 minute at **6,000 x g (~8,000 RPM)**.

   **Note:** Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

b. Discard the flowthrough and reassemble the column with its collection tube.
c. Repeat step **3a** to wash column a second time.
d. Discard the flowthrough and reassemble the spin column with its collection tube.
e. Spin the column for 2 minutes at **14,000 x *g* (~14,000 RPM)** in order to thoroughly dry the resin. Discard the collection tube.

**4. Nucleic Acid Elution**

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 100 µL of **Elution Buffer** to the column.
c. Centrifuge for 2 minutes at **6,000 x g (~8,000 RPM).** Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.

## B. *B. cinerea* PCR Assay Preparation

**Notes:**

- Before use, suitable amounts of all PCR components should be completely thawed at room temperature, vortexed and centrifuged briefly.
- The amount of BC 2X Detection PCR Master Mix and Control 2X PCR Master Mix provided is enough for up to 32 PCR reactions (24 sample PCR, 4 positive control PCR and 4 no template control PCR).
- For each sample, one PCR reaction using the BC 2X Detection PCR Mastermix and one PCR reaction using Control 2X PCR Mastermix should be set up in order to have a proper interpretation of the results.
- For every PCR run, one reaction containing BC Positive Control and one reaction as no template control must be included for proper interpretation of results.

- The recommended minimum number of DNA samples tested per PCR run is 6.
- Using a lower volume from the sample than recommended may affect the sensitivity of *B. cinerea* Limit of Detection.

1. Prepare the PCR reaction for sample detection (Set #1, using **BC 2X Detection PCR Mastermix**) and the PCR reaction for control detection (Set #2, using **Control 2X PCR Mastermix**) as shown in Table 1 below. The recommended amount of sample DNA to be used is 2.5 µL. However, a volume between 1 and 5 µL of sample DNA may be used as template. Ensure that one *B. cinerea* detection reaction and one control reaction is prepared for each DNA sample. Adjust the final volume of the PCR reaction to 20 µL using the Nuclease-Free Water provided.

**Table 1. PCR Assay Preparation**

| PCR Components | Volume Per PCR Reaction |
|---|---|
| **BC 2X Detection PCR Master Mix Or Control 2X PCR Master Mix** | **10 µL** |
| **Sample DNA** | **2.5 µL** |
| **Nuclease-Free Water** | **7.5 µL** |
| ***Total Volume*** | **20 µL** |

2. For each PCR set, prepare **one** positive control PCR as shown in Table 2 below:

**Table 2. PCR Positive Control Preparation**

| PCR Components | Volume Per PCR Reaction |
|---|---|
| **BC 2X Detection PCR Master Mix Or Control 2X PCR Master Mix** | **10 µL** |
| **BC *Positive Control (PosC)*** | **10 µL** |
| ***Total Volume*** | **20 µL** |

3. For each PCR set, prepare **one** no template control PCR as shown in Table 3 below:

**Table 3. PCR Negative Control Preparation**

| PCR Components | Volume Per PCR Reaction |
|---|---|
| **Bc 2X Detection PCR Master Mix Or Control 2X PCR Master Mix** | **10 µL** |
| ***Nuclease-Free Water*** | **10 µL** |
| ***Total Volume*** | **20 µL** |

## C. *B.cinerea* PCR Assay Programming

1. Program the thermocylcer according to the program shown in Table 4 below.
2. Run one step PCR.

**Table 4. *B.cinerea* Assay Program**

| PCR Cycle | Step | Temperature | Duration |
|---|---|---|---|
| *Cycle 1* | Step 1 | 95°C | 3 min |
| *Cycle 2 (35x)* | Step 1 | 94°C | 15 sec |
| | Step 2 | 60°C | 15 sec |
| | Step 3 | 72°C | 30 sec |
| *Cycle 3* | Step 1 | 72°C | 5 min |
| *Cycle 4* | Step 1 | 4°C | ∞ |

## D. *B.cinerea* PCR Assay Results Interpretation

1. For the analysis of the PCR data, the entire 15-20 µL PCR Reaction should be loaded on a 1X TAE 1.5% Agarose DNA gel along with 10 µL of Norgen's DNA Marker (provided).
2. The PCR products should be resolved on the 1X TAE 1.5% Agarose gel at 150V for 30 minutes (Gel running time will be vary depending on an electrophoresis apparatus).
3. Sample results are provided below:

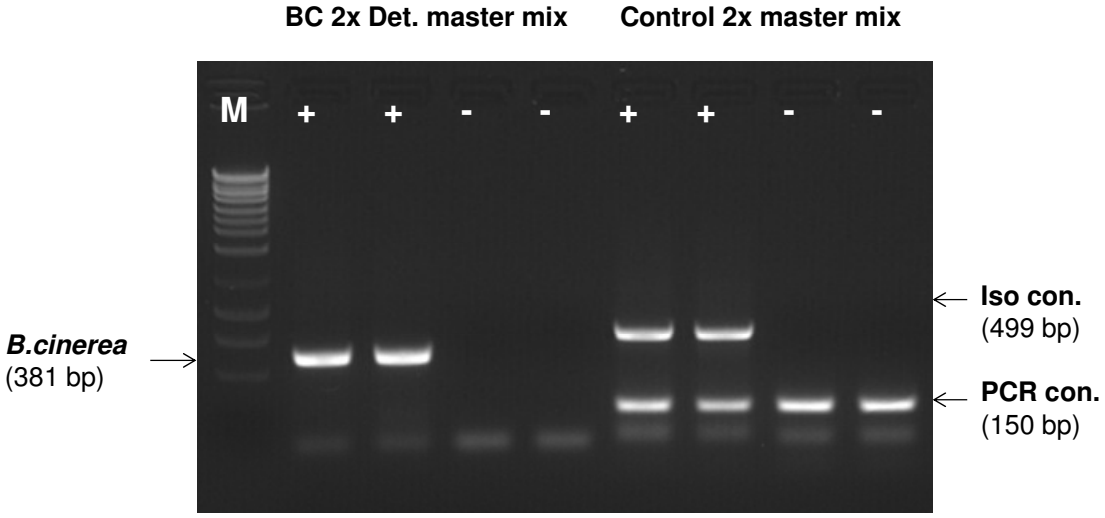


**Figure 1. Sensitivity of Detection using the *Botrytis cinerea* PCR Detection Kit.** A representative 1X TAE 1.5% agarose gel showing the amplification of *B.cinerea* and the amplification of Isolation Control and PCR Control under different conditions using the BC 2x Detection Master Mix and Control 2X PCR Mastermix. The size of the *B.cinerea* amplicon is 381 bp, the size of the Isolation Control amplicon is 499 bp, and the size of the PCR Control amplicon is 150 bp, as represented by the provided DNA Marker (M). Positive (+) samples showed an expected PCR amplicon with the BC 2x Detection Mastermix and Control 2x Master mix, suggesting that the specific *B. cinerea* detection, DNA isolation and the PCR reaction was successful. All negative (-) samples did not give any PCR amplification.

**Table 5. Interpretation of PCR Assay Results**

| Input Type | Target reaction | Control Reaction | | Interpretation |
|---|---|---|---|---|
| | ***B. cinerea* Target Band (381 bp)** | ***IsoC* Band (499 bp)** | ***PCRC* Band (150 bp)** | |
| Positive Control | X | X | X | Valid |
| Negative Control | | | X | Valid |
| Sample | X | X | X | Positive |
| Sample | | X | X | Negative |
| Sample | | | X | Re-test |
| Sample | | | | Re-test |
| Sample | | X | | Negative |
| Sample | X | | X | Positive |
| Sample | X | X | | Positive |
| Sample | X | | | Re-test |

****** For results obtained that are not covered in Table 5 above, please refer to the Troubleshooting Section.

## E. *B.cinerea* PCR Assay Specificity and Sensitivity

- The specificity of Norgen's *Botrytis cinerea* PCR Detection Kit is first and foremost ensured by the selection of the *B.cinerea* specific primers, as well as the selection of stringent reaction conditions. The *B.cinerea* specific primers were checked for possible homologies to GenBank published sequences by sequence comparison analysis and published strains.

## F. Linear Range

- The linear range of Norgen's *Botrytis cinerea* PCR Detection Kit was determined by analysing a dilution series of a *B. cinerea* quantification standards ranging from 100 ag to 1 pg.
- Each dilution has been tested in replicates (n = 4) using Norgen's *B. cinerea* PCR Detection Kit on a 1X TAE 1.7% agarose gel.
- The linear range of Norgen's *B.cinerea* PCR Detection Kit has been determined to cover concentrations from 100 ag to 1 ng
- Under the conditions of the Norgen's *B. cinerea* DNA Isolation procedure, Norgen's *B. cinerea* PCR Detection Kit covers a linear range from 100 copies to 1 x $10^6$ copies.

## Frequently Asked Questions

**1. How many samples should be included per PCR run?**

- Norgen's *Botrytis cinerea* PCR Detection Kit is designed to test 24 samples. For every 6 samples, a non-template control (Nuclease Free Water) and a Positive Control must be included. It is preferable to pool and test 6 samples at a time. If not, the provided Positive Control is enough to run 3 samples at a time.

**2. How can I interpret my results if neither the PCR control nor the Isolation Control (*IsoC*) amplifies?**

- If neither the PCR control nor the Isolation Control (*IsoC*) amplifies, the sample must be re-tested. If the positive control showed amplification, then the problem occurred during the isolation, where as if the Positive control did not amplify, therefore the problem has occurred during the setup of the PCR assay reaction.

**3. How should it be interpreted if only the PCR control showed amplification but neither the *B. cinerea* target nor the Isolation control amplified for a sample?**

- This indicates a poor isolation. The isolation procedure must be repeated.

**4. How should it be interpreted if only the Isolation Control (*IsoC*) was amplified in a sample?**

- The sample tested can be considered as *Botrytis cinerea* negative.

**5. How should it be interpreted if the PCR control and the *B. cinerea* target showed amplification in a sample?**

- The sample tested can be considered positive. It could happen when too much template was added to the reaction.

**6. How should it be interpreted if only the *B. cinerea* target and the PCR control were amplified in a sample?**

- The sample tested can be considered as *Botrytis cinerea* positive.

**7. How should it be interpreted if only the *B. cinerea* target was amplified in a sample?**

- It is recommended that the isolation is repeated.

**8. How should it be interpreted if only the PCR control and the Isolation control showed amplification in a sample?**

- The sample tested can be considered negative

**9. What if I forgot to do a dry spin after my third wash?**

- Your first DNA elution will be contaminated with the Wash Solution. This may dilute the DNA yield in your first elution and it may interfere with the PCR detection, as ethanol is known to be a PCR inhibitor.

**10. What if I forgot to add the Isolation Control (*IsoC*) during the isolation?**

- It is recommended that the isolation is repeated.

| Related Products | Product # |
|---|---|
| Plant/Fungi DNA Isolation Kit | 26200 |
| Plant RNA/DNA Purification Kit | 24400 |
| Plant/Fungi Total RNA Purification Kit | 25800 |

**Technical Assistance**

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's *Botrytis cinerea* PCR Detection Kit or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362 or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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