MGC
2025
v2

MEDICINAL GENOMICS®

# PathoSEEK® Powdery Mildew Detection Assay v3

QUICK GUIDE V2

## Test Kit Information

PathoSEEK® Powdery Mildew qPCR Detection Assay v3 Kit - P/N 420542 (200 reactions)

| Component Name | Qty Provided | Storage Conditions |
|---|---|---|
| PathoSEEK® Amplification Mix<br>*Includes 2 tubes nuclease free water for resuspension* | 4 Vials (50 rxns/each) | RT / -20 ºC* |
| PathoSEEK® Powdery Mildew Detection Assay v3 | 1 Tube (200 µL) | -20 ºC |

Note: Actual fill volumes include overage

*The PathoSEEK Amplification Mix can be stored lyophilized at Room Temperature for up to 2 years. Once re-hydrated it must be stored at -20 ºC for up to 3 months.

Additional Required Components Not Included in Kits:

| Item P/N | Item Name | Qty Provided | Storage Conditions |
|---|---|---|---|
| 420216 | Powdery Mildew Positive Control | 1 Tube (50 µL) | -20 ºC |
| 100164 | 96 Well PCR Plate | Case of 25 | RT |
| 100177 | Optical Adhesive Seals | Case of 100 | RT |

**DNA Lysis/Purification**

For Plant Sampling and DNA lysis or purification instructions see the Sample Preparation Guide which should be followed *before* setting up the Powdery Mildew qPCR reactions.

**qPCR Setup:**

1. Prepare Assay Master Mix

   qPCR Reagent Volumes

| Reagents | 1 Reaction |
|---|---|
| Amplification Mix | 10 µL |
| Water | 4 µL |
| Detection Assay | 1 µL |
| **Total** | **15 µL** |

   a. Prepare enough master mix for your samples plus two controls (positive and NTC). Add 10% overage to the master mix components to account for pipetting and dead volumes.

2. Prepare Positive Control Dilution
   a. Dilute the stock assay positive control 1:10 with nuclease free water. 9 µL water, 1 µL positive control, vortex and spin down.
3. Transfer samples and master mix to PCR plate
   a. Transfer 5 µL of each sample, 5 µL of diluted assay positive control, and 5 µL of water to separate wells of a qPCR plate.
   b. Transfer 15 µL of freshly prepared qPCR Assay Master Mix to each well and slowly tip mix. Avoid adding bubbles to the mixture.
4. Seal plate, spin in plate centrifuge and load on qPCR instrument.
5. Powdery Mildew - qPCR cycling parameters:
   a. Hot Start at 95 ºC for 5 minutes

      Followed by 40 cycles of:
   b. 95 ºC for 15 seconds
   c. 65 ºC for 1 min and 30 seconds.

   d. Plate Read
6. Start the run.
7. When the run is complete, the plate can be discarded.
8. Proceed to data analysis.

**Data Analysis:**

| Powdery Mildew Assay | Cq Value | Fluor | Negative Control (Cq) | Cq Threshold |
|---|---|---|---|---|
| Positive Powdery Mildew Result | < 40 | FAM | No Value | Presence/Absence |
| | | | | |
| Internal Cannabis Control* | <35 | HEX | *Internal control verifies the presence or absence of cannabis DNA | |
| Assay Positive Control | <35 | FAM | | |

1. Positive and No Template (NTC) Controls - Confirm Assay Positive control well and assay NTC well results are as expected.
   a. Assay positive control should have a Cq value ≤ 35 for FAM.
      i. No HEX signal should be observed in the control wells
         1. If HEX signal is observed a Cq of >35 is acceptable.
   b. Assay NTC should have no Cq value for FAM.
      i. No HEX signal should be observed in the control wells
         1. If HEX signal is observed a Cq of >35 is acceptable.
   c. Confirm Cq values against amplification plots.
2. Sample Analysis
   a. Internal Cannabis Control (HEX)
      i. HEX signals in sample wells should be ≤ 35
   b. Powdery Mildew Positive samples (FAM)
      i. FAM amplification which results in a Cq value ≤ 40
   c. Confirm Cq values against amplification plots.

REVISION HISTORY

| Version | Date | Description |
|---|---|---|
| v1 | November 2024 | Quick Guide Format |
| v2 | October 2025 | Update assay version, amplification mix, and kit |

DISCLAIMER

This test was developed, and its performance characteristics determined by Medicinal Genomics Company, for laboratory use. Any deviations from this protocol are not supported by MGC. The results may vary based on laboratory conditions.

LIMITED USE LABEL LICENSE

This product is covered by at least one or more claims of US patent applications, which are exclusively licensed to Medicinal Genomics Corporation. This product is sold strictly for the use of the buyer, and the buyer is not authorized to transfer this product [or any materials made using this product] to any third party.