Sample Preparation Guide for PathoSEEK® qPCR Plant Pathogen Detection Assays and FemINDICAtor® qPCR Plant Sex Detection Assay

User Guide

This guide describes how to prepare plant samples for nucleic acid (DNA/RNA) extraction or purification from various matrices.

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Introduction

PathoSEEK® qPCR and RT-qPCR Plant Pathogen Detection Assays are designed to detect nucleic acid sequences that are unique to certain plant pathogens, such as Hop Latent Viroid (HLVd), Powdery Mildew, Botrytis, Russet Mites, Fusarium, and more. The FemINDICAtor qPCR Plant Sex Detection Assay is designed to detect a DNA sequence unique to the cannabis/hemp y-chromosome.

This user guide describes the process for obtaining DNA or RNA from cannabis/hemp leaves, roots or water. The genomic material can then be tested using PathoSEEK® qPCR/RT-qPCR Plant Pathogen Detection Assays or the FemINDICAtor qPCR Plant Sex Detection Assay.

Instructions for qPCR setup and data analysis for each individual assay can be found separately in our Quick Guide menu. Visit medicinalgenomics.com/product-literature/

Note: For Microbial Safety Testing (Compliance) Assays, please refer to the individual User Guides for each at medicinalgenomics.com/product-literature/

Sample Prep Options

1. Quick Lysis - P/N 420240
    a. Nucleic Acid Extraction Solution — packaged in 12 x 0.2 mL PCR 8-tube strips with attached optical cap strip in a 96 well tube rack (Store at -15 to -20°C). Expires 1 Year from Date of Manufacture.
    b. Sample punch grommets included.
2. Pure Prep - P/N 420031
    a. Nucleic Acid Purification Solution - packaged in 12 x 0.2 mL PCR 8-tube strips with attached optical cap strip in a 96 well tube rack (Store at 4°C). Expires 1 Year from Date of Manufacture.
    b. Sample punch grommets included.

Supplies, Reagents, and Equipment

1. Thermal Cycler —Capable of maintaining 95ºC and heated lid or similar (Eppendorf ThermoStat™ C Item #4053-8327 + Eppendorf SmartBlock™ Item #4053-6006 (PCR96) + Eppendorf ThermoTop Item #4053-8003)
2. Dual rotor personal microcentrifuge, (USA Scientific #2641-0016)
3. Table Top Vortex Genie (Scientific Industries #SI-0236 or Similar)
4. Refrigerator—Capable of maintaining 4ºC
5. Lab Freezer—Capable of maintaining -20ºC
6. 96-well Plate Magnet - Medicinal Genomics P/N 420202 (**Pure Prep only**)
7. 96-Well Optical qPCR plate - Medicinal Genomics P/N 100164 (**Pure Prep only**)
8. Applied Biosystems MicroAmp Optical Film Compression Pad, Fisher Scientific, #43-126-39 (for use with Agilent Aria MX only)
9. 70% Ethanol—Medicinal Genomics P/N 420030
10. Multi-channel pipette P20, P200 (optional) **(Pure Prep Only)**
11. Single channel pipette P20, P200 **(Pure Prep Only)**
12. Filtered pipette tips for, P20, P200 **(Pure Prep Only)**
13. Laboratory Gloves (USA Scientific, # 4904-3300 or similar)
14. Permanent Marker (Sharpie)
15. 1.5 mL Sample Tubes (Optional)
16. Plant Stakes (Optional)
17. Toothpicks for punching out leaf punches (can use pipette tips as well)
18. 10% bleach
19. 50 mL Reagent Reservoirs (USA Scientific, #1930-2535 or similar)
20. 50 mL centrifuge tubes (Thomas Scientific, #1163X44 or similar)
21. Strip tubes (USA Scientific, #1402-2300)
22. PCR Grade Nuclease Free Water—Medicinal Genomics P/N 420184
23. SCCG Internal Cannabis Control - Medicinal Genomics P/N 420326 (for water testing)

24. IC (Internal Control) - Medicinal Genomics P/N 420337 (for water testing)

Safety Precautions

Users should observe good lab practices and safety precautions when sampling. Wear protective gloves, lab coats, eye/face protection as indicated by your quality system. It is the responsibility of each laboratory to handle waste and effluents processed according to their nature and degree of hazardousness and to treat and dispose of them in accordance with applicable local, state, and federal regulations.

Hazard Statement: 70% Ethanol

a. Highly flammable liquid and vapor May cause respiratory irritation
b. May cause drowsiness or dizziness Causes damage to organs
c. May cause damage to organs through prolonged or repeated exposure
d. Please refer to the Safety Data Sheet (SDS) for more information and proper disposal

Plant Tissue Sampling Recommendations and Best Practices

Choose the right tissue type for your target

Many different phytopathogens are known to infect the roots and leaves of cannabis and hemp plants both indoors and outdoors. Therefore, sampling decisions should be based on the phytopathogen of interest. See the table below for our recommendations.

Table I: Recommended Input Type and Lysis Method by Assay

Assay Target	Recommended Input Type	Recommended Lysis Method
Plant Sex (FemINDICAtor)	Leaf	Quick Lysis
Hop Latent Viroid	Root / Leaf/ Water	Pure Prep
Pan *Fusarium*	Root/ Water	Pure Prep / Quick Lysis
Fusarium *oxysporum*	Root/ Water	Pure Prep / Quick Lysis
Pan *Pythium*	Root/ Water	Pure Prep / Quick Lysis
Botrytis	Leaf	Quick Lysis
Powdery Mildew	Leaf	Quick Lysis
Russet Mites	Leaf	Quick Lysis
Tobacco Mosaic/ Beet Curly Top/ Lettuce Chlorosis/ Cannabis Cryptic Virus	Multiple/ Water	Pure Prep / Quick Lysis

- **FemINDICAtor** is used to determine the sex of cannabis and hemp seedlings, therefore leaf is the most common tissue type used for that assay. However, the genetic material responsible for plant sex determination is in all tissue types, and any tissue could technically be used for FemINDICAtor.
- Roots typically produce the strongest qPCR signal if a plant is infected with **Hop Latent Viroid** (HLVd), and in some cases, roots have tested positive for HLVd, while leaves from the same plant tested negative. Consider sampling multiple locations of the plant for a more comprehensive assessment of infection.
- Root and stem tissues are a common area for ***Fusarium*** **and** ***Pythium*** species. which can cause vascular wilt and damping off respectively.

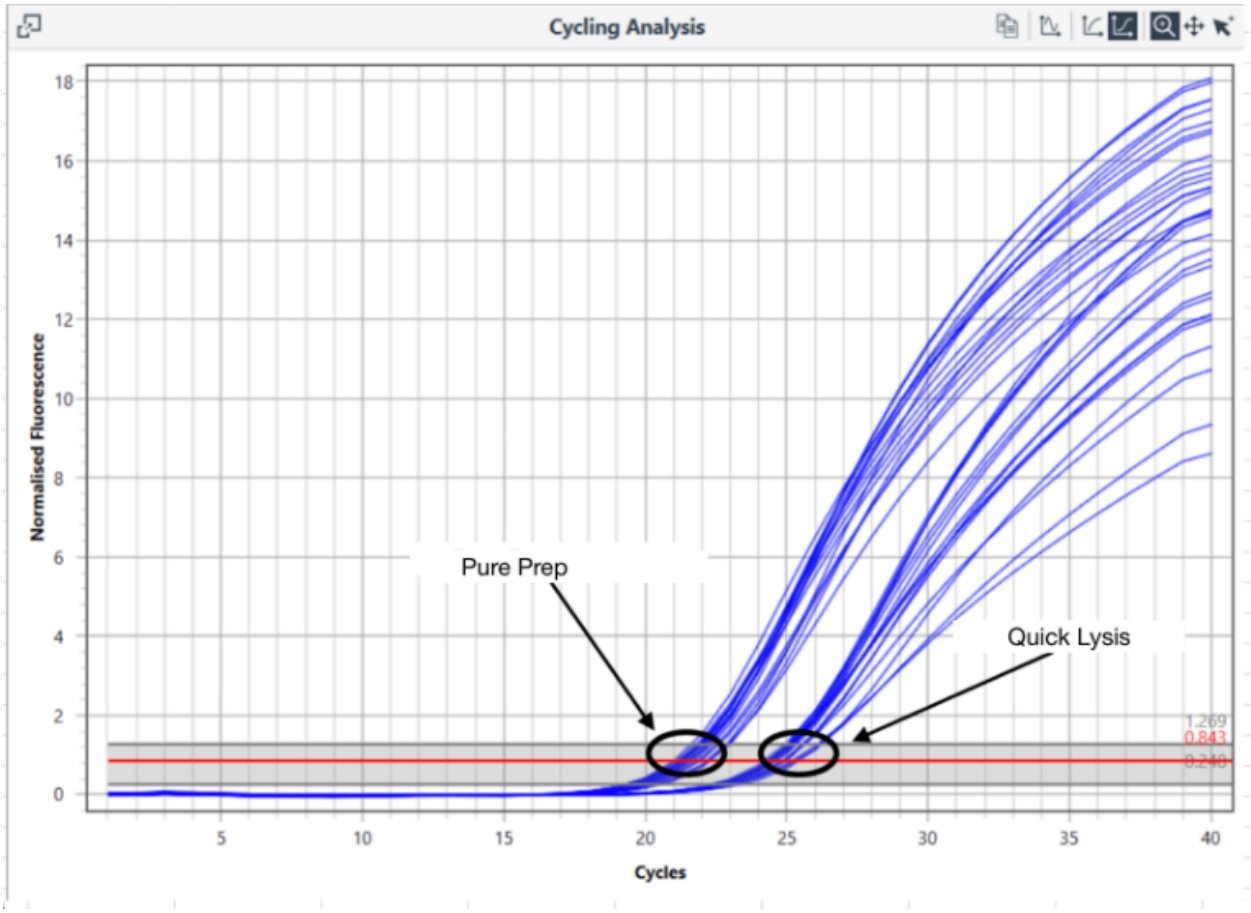
- ***Botrytis cinerea*** destroys cannabis and hemp inflorescence; however, it can originate from endophytic colonization released from leaves or stems.
- **Powdery Mildew** infection can be visibly seen on leaves once the proper conditions occur, however, infection could arise from pre-symptomatic colonization of plant tissue.
- **Tobacco Mosaic Virus, Beet Curly Top Virus, Cannabis Cryptic Virus, and Lettuce Chlorosis Virus** have been reported in cannabis; however, our team has had limited access to infected samples. Therefore, we can't offer authoritative guidance on which tissue will provide the best results. We recommend sampling multiple locations of the plant as this will result in a more comprehensive assessment of infection.

Choose the right lysis for your goals

There are two different lysis methods that are compatible with PathoSEEK® qPCR and RT-qPCR Plant Pathogen Detection Assays.

- Quick Lysis: a simple boil-based prep, which requires no manual pipette steps.
- Pure Prep: a bead-based purification method that concentrates the DNA and RNA present in the sample and eliminates PCR inhibitors.

Internal experiments comparing Pure Prep to Quick Lysis showed that Pure Prep recovered 10 times more HLVd RNA from infected root samples than Quick Lysis.



When to Use Pure Prep

If your goal is to detect a plant pathogen as early as possible, when genomic copies are very low, then Pure Prep is the best option. Pure Prep recovers more genetic material from the sample and reduces PCR inhibitors, so the assay can more easily detect low-level infections.

Service providers who advertise extremely low limits of detection are likely performing methods that involve a DNA or RNA cleanup (purification and concentration of nucleic acids) like the Pure Prep method to remove inhibitors from the sample prior to RT-qPCR or qPCR. Therefore, to obtain results that are comparable to those services, we recommend using Pure Prep.

We recommend Pure Prep be used to purify DNA and RNA from root samples, due to inhibitors that are commonly found in soil, soilless media, and hydroponic systems.

When to Use Quick Lysis

Quick Lysis requires no manual pipette steps, making it a good option for growers who do not have laboratory experience. It is also a more scalable option for customers who are screening many plants on a regular basis.

For assays like FemINDICAtor, which look for the presence of the Y chromosome in cannabis DNA, sensitivity is not an issue. The amount of y-chromosome DNA is fairly constant, therefore, the DNA recovered from Quick Lysis is more than sufficient.

Do not pool multiple punches of leaf or root into Quick Lysis or Pure Prep wells

Doing so will overload the lysis/purification and therefore the RT-qPCR or qPCR reaction. Multiple punches have been shown to inhibit the assays.

Items needed for sampling

- Grommets (included with Quick Lysis or Pure Prep)
- Disposable gloves
- Spray bottle of 10% bleach
- Scissors
- Cup/beaker of 10% bleach solution
- Kim wipes or Paper towels
- 1.5 mL Sampling Tubes
- Plant Stakes
- Marker
- Nuclease Free Water (root samples only)
- 50 mL Reagent Reservoir or 50 mL centrifuge tubes (root samples only)

Clearly label plants

Make sure to clearly label the plant you are sampling in such a way that you can easily associate it with the sample you collect and ultimately the qPCR (or RT-qPCR) result that is associated with each sample.

Sterilize hands and tools between samples

Plant pathogens are easily spread via mechanical transmission, so it is vital to sterilize any equipment that comes into contact with the plant's vasculature.

- Soak trimming tool in 10% bleach solution for 60 seconds.
- Spray 10% bleach solution onto any tool parts that were not submerged in bleach solution.
- Use a new pair of gloves for each sample. **Pro Tip:** Put on two pairs of gloves and change the outer set between plants or spray your hands with 10% bleach solution in between each sample.
- Use a different grommet for each sample.
- Use fresh Nuclease Free water for rinsing each root sample.

Root Sampling

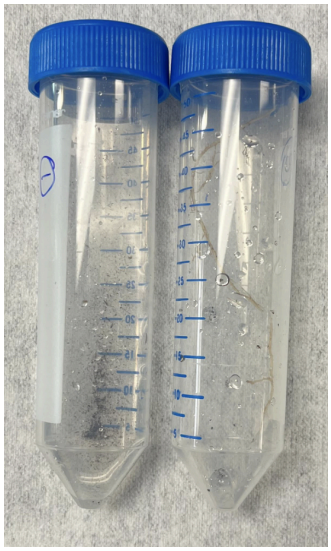
We recommend using Pure Prep to purify DNA and RNA from root samples.

1. Put on a new pair of sterile gloves.
2. Collect the root samples from the plants you plan to test, and place them on a clean, dry surface (Kim wipe or paper towel is recommended). Be sure to change gloves when handling different samples to avoid cross-contamination.
    a. For plants grown in soil, coco coir, or peat-based media, lightly brush the topsoil of the plant to reveal the surface root, and pull gently on the root to break it away from the root mass. Only a small amount of root tissue is needed (5-10 cm).

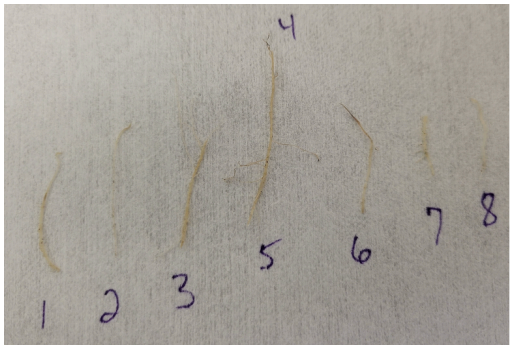
    b. For plants grown in hydroponic systems or clones in rooting plugs, use sterile scissors to cut small pieces of exposed root tissue. Only a small amount of root tissue is needed (5-10 cm).
    c. Place the sampled root on a clean paper towel or if sampling a large volume of samples place the root sample in a clearly labeled 1.5 mL sample tube.
3. Remove Pure Prep strip tube(s) and allow them to thaw.
4. Once thawed, quick spin the Pure Prep strip tube(s) using a mini centrifuge with strip tube adapter to bring all contents to the bottom of the tube(s).

5. Label the wells of the Pure Prep Solution strip tube(s) to match the associated plant labels.
6. Fill 50 mL Reagent Reservoir with Nuclease free water.
7. Place root sample(s) from one plant in the nuclease-free water and remove all excess soil or growing media.

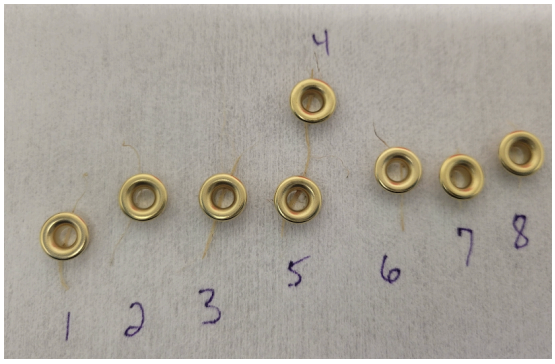

8. For larger sample sizes, roots may be cleaned in 50 mL tubes with Nuclease free water. Vortex to loosen soil from roots.



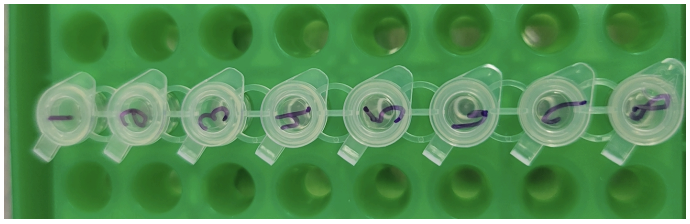
9. Place the roots on a clean dry surface. A Kim wipe or paper towel is recommended.

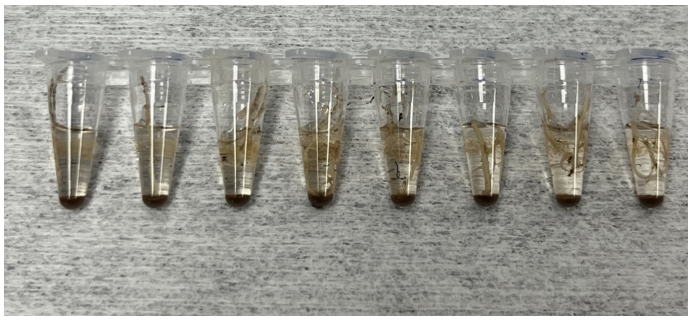


10. Use a grommet to sample the root. Be sure to use a different grommet for each sample to avoid any cross-contamination.



11. Place the punched root material into a labeled Pure Prep strip tube well, and close the cap.
12. Repeat steps 6-11 for each sample being tested, using new gloves, new grommets, and fresh water for each root sample.

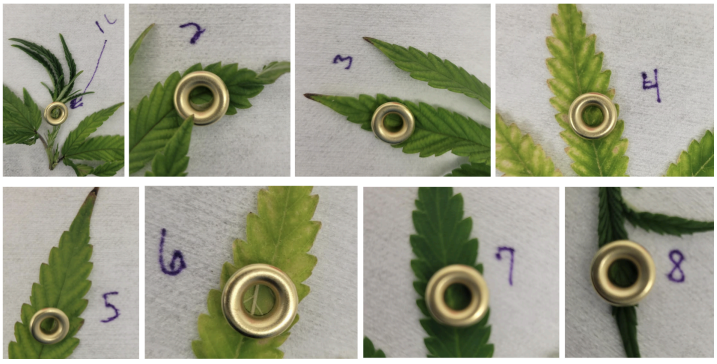




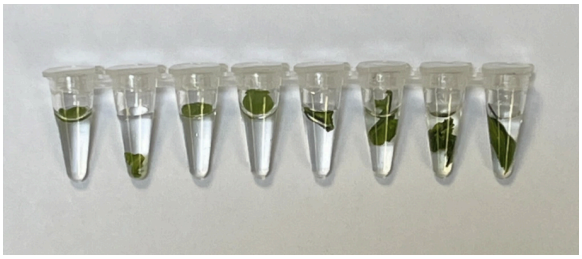
13. Once all sampling is complete, proceed to the Pure Prep instructions - our recommended lysis method for root samples.

Leaf Sampling

1. Put on a new pair of sterile gloves.
2. Collect the leaf samples from the plants you plan to test, and place them on a clean, dry surface (Kim wipe or paper towel is recommended). If sampling a large volume of samples place the leaf tissue sample in a clearly labeled 1.5 mL sample tube. Be sure to change gloves when handling different samples to avoid cross-contamination.
3. If leaves are known to have been treated with foliar sprays or something similar, wipe off leaves with a Kim Wipe dampened with nuclease free water before sampling.
4. If using Quick Lysis, allow the contents to thaw.
5. Quick spin strip tube(s) using a mini centrifuge with strip tube adapter, bringing all contents of the solution to the bottom of the tube(s).
6. Label the wells of the Pure Prep or Quick Lysis Solution strip tube(s) to match the associated plant labels.
7. Use a grommet to sample the leaves. Press the grommet down into the midrib of the leaf on a sterile surface and rotate to remove a small section. Be sure to use a different grommet for each sample to avoid any cross-contamination.



8. Remove the cap from the labeled Pure Prep or Quick Lysis strip tube well and place the leaf punch into the top of the strip tube. Discharge leaf punch with a toothpick or pipette tip. Use a fresh toothpick or pipette tip for every leaf punch.



9. Close the strip tube cap and discard the leaf punch grommet.
10. Repeat steps 6-8 for each sample being tested, changing gloves after each leaf sampling.
11. Once finished with leaf sampling, proceed to either the Quick Lysis Instructions or the Pure Prep Instructions, depending on which method you are using.

Water Sampling

1. Remove Pure Prep strip tube(s) and allow them to thaw.
2. Quick spin the Pure Prep strip tube(s) using a mini centrifuge with strip tube adapter to bring all contents to the bottom of the tube(s).
3. Pipette tip mix the Pure Prep tubes 15 times then transfer 75 µL Pure Prep to a fresh strip tube.
4. Add 75 µL of water to the 75 µL Pure Prep tubes and tip mix 15 times to combine.
5. Add 5 µL 1:50K diluted Internal Control (see below Table 2 for internal control information)
6. Proceed to Pure Prep Instructions - Water.

Table 2: Internal Control type by assay

Assay Target	Internal Control
Plant Sex (FemINDICAtor)	SCCG
Hop Latent Viroid	IC
Pan *Fusarium*	IC
Fusarium *oxysporum*	SCCG
Pan *Pythium*	IC
Botrytis	SCCG
Powdery Mildew	SCCG
Russet Mites	IC
Tobacco Mosaic/ Beet Curly Top/ Lettuce Chlorosis/ Cannabis Cryptic Virus	SCCG

Quick Lysis Instructions

1. Quick spin the samples in a centrifuge to ensure all plant material is in contact with the Quick Lysis solution and there is no Quick Lysis Solution in the lid.
2. Place strip tube(s) onto the Thermal Cycler.
3. If using an Agilent Aria MX, place a compression pad over tubes to prevent opening of caps during lysis. This can lead to evaporation and cross contamination between strip tubes.
4. Run with the following parameters:
    a. 65 ºC, 6 minutes
    b. 95 ºC, 2 minutes
    c. 4 ºC or Room temperature
5. Remove tubes from the thermal cycler and quick spin tube(s) in microcentrifuge with strip tube rotor to remove condensation from caps before opening them
6. Proceed to qPCR Setup and Data Analysis
    a. If using the Mic and Myra Semi-automated qPCR Platform, refer to the "Mic and Myra User Guide", available at medicinalgenomics.com/product-literature
    b. If using a standard thermal cycler, refer to the appropriate Quick Guide, available at medicinalgenomics.com/product-literature

Pure Prep Instructions - Plant Tissue

1. Quick spin the samples in a centrifuge to ensure all plant material is in contact with the Pure Prep solution and there is no Pure Prep Solution in the lid.
2. Place Pure Prep strip tube(s) onto the Thermal Cycler. If using an Agilent AriaMX, cover tubes with a compression pad to prevent opening of caps during lysis. This can lead to evaporation and cross contamination between strip tubes.
3. Run using the following parameters:
    a. 92 ºC, 8 minutes
    b. 4 ºC or Room temp
4. Remove Pure Prep strip tube(s) from Thermal Cycler and quick spin in microcentrifuge to remove condensation from caps before opening them.
5. Tip mix each well 5 times or until beads are fully resuspended with a pipette set to 75 µL.
6. Transfer 75 µL of lysate into a fully skirted PCR Plate.
7. With fresh tips, add 75 µL of 70% ethanol to each lysate and tip mix sample lysates and ethanol 5 times to thoroughly mix.
8. Let mixture sit at room temperature for 5 minutes
9. Move the PCR plate to 96-well Plate Magnet for 5 minutes, allowing the beads to form rings.
10. Remove and discard all liquid, avoiding the beads. Do NOT discard beads.
11. Leave the PCR plate on the magnet and perform two 70% ethanol washes:
    a. Transfer 150 µL of 70% ethanol to each sample well
    b. Wait 30 seconds and remove and discard ethanol
    c. Repeat wash a second time
    d. Remove all ethanol and discard
12. Allow the beads dry for 5-10 minutes at room temperature.

13. Remove the PCR plate from the magnet and resuspend beads in 20 µL Nuclease free water by tip mixing. The nuclease free water may need to be dispensed onto the bead ring during mixing to aid in this resuspension.
14. Place the PCR plate back on the magnet to separate DNA and RNA material from beads. Beads will form rings in less than 1 minute. Samples are now ready for RT-qPCR or qPCR setup.
15. If not moving forward with PCR setup right away, seal and store plate at 4℃. For long term storage, transfer the eluted DNA off the beads into new fresh wells or a new plate. Seal the plate and store at - 20℃
16. Proceed to qPCR Setup and Data Analysis
    a. If using the Mic and Myra Semi-automated qPCR Platform, refer to the "Mic and Myra User Guide", available at medicinalgenomics.com/product-literature
    b. If using a standard thermal cyclers, refer to the appropriate Quick Guide, available at medicinalgenomics.com/product-literature

Pure Prep Instructions - Water

1. Quick spin the samples in a centrifuge to ensure that there is no Pure Prep Solution in the lid.
2. Place Pure Prep strip tube(s) onto the Thermal Cycler. If using an Agilent AriaMX, cover tubes with a compression pad to prevent opening of caps during lysis. This can lead to evaporation and cross contamination between strip tubes.
3. Run using the following parameters:
    a. 92 ºC, 8 minutes
    b. 4 ºC or Room temp
4. Remove Pure Prep strip tube(s) from Thermal Cycler and quick spin in microcentrifuge to remove condensation from caps before opening them.
5. Pipette tip mix the tubes.
6. Transfer 100 µL of the lysed sample to a well of a 96 well PCR Plate
7. Add 100 µL of 70% Ethanol to the 100 µL of lysed sample and tip mix
8. Allow samples to incubate at room temperature for 5 minutes.
9. Place the qPCR plate onto a 96 well magnet plate for 5 minutes.
10. Remove 200 µL supernatant with qPCR plate still on the magnet plate, avoiding the beads. Do NOT discard beads.
11. Perform two 70% ethanol washes
    a. Transfer 200 µL 70% ethanol to each sample well
    b. Wait 30 seconds and remove and discard ethanol
    c. Repeat wash a second time
    d. Remove all ethanol and discard
12. Allow the beads to dry at room temperature for up to 15 minutes.
13. Remove the qPCR plate from the magnet and add 15 µL of nuclease free water to each sample. Pipette tip mix to combine nuclease free water and beads.
14. Place the qPCR plate back on the magnet for 1 minute before proceeding to qPCR setup.

15. If not moving forward with PCR setup right away, seal and store plate at 4℃. For long term storage, transfer the eluted DNA off the beads into new fresh wells or a new plate. Seal the plate and store at - 20℃
16. Proceed to qPCR Setup and Data Analysis
    a. If using the Mic and Myra Semi-automated qPCR Platform, refer to the "Mic and Myra User Guide", available at medicinalgenomics.com/product-literature
    b. If using a standard thermal cyclers, refer to the appropriate Quick Guide, available at medicinalgenomics.com/product-literature

REVISION HISTORY

Version	Date	Description
v1	November 2023	Quick Lysis and Pure Prep Product Launch
v2	December 2024	• Added sampling instructions for testing water samples • Added requirement to use compression pad on strip tubes for lysis steps
v3	April 2025	• Added instructions for testing leaves with foliar sprays or similar • Updated Russet Mite Assay to v2 (targets IC instead of SCCG)
v4	August 2025	• Moved Internal Control types to Water Testing section • Added hazard statement for ethanol • Added quick spin for Quick Lysis samples before heating

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.

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