

**PathoSEEK[®] Detection Assays with SenSATIVAx[®] DNA
Purification and Grim Reefer[®] Free DNA Removal for Bacterial
Targets**

**Supplemental User Guide describing the addition of the Grim Reefer Free DNA
Removal Steps**

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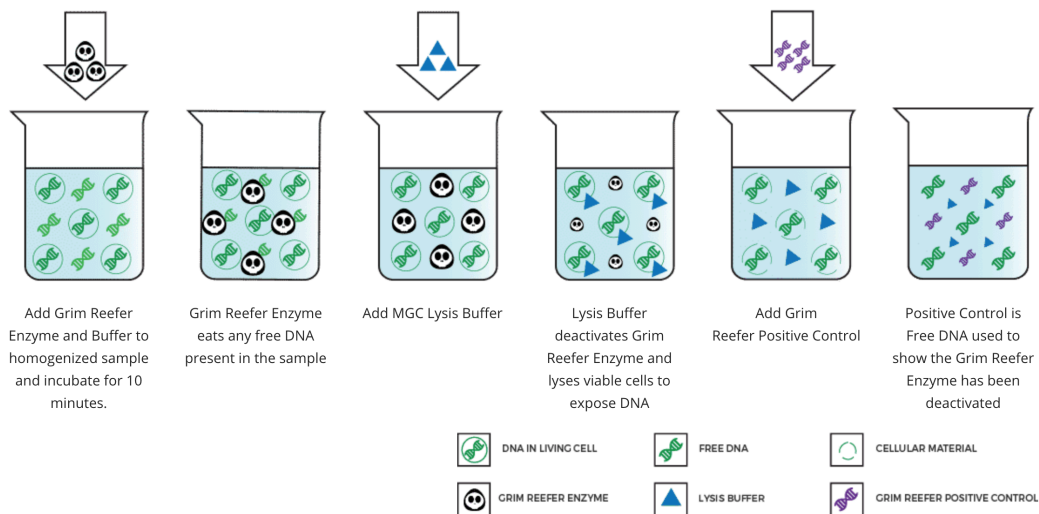
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Introduction

This supplemental guide describes the process for using Grim Reefer® Free DNA Removal Kit reagents in conjunction with SenSATIVAx® DNA Purification Kits and PathoSEEK® qPCR Detection Assays for bacterial targets, including *Salmonella spp.*, *E. coli*, STEC, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. For sample preparation and data analysis instructions, refer to the appropriate User Guide at medicinalgenomics.com/product-literature/.

Process Overview

SenSATIVAx® DNA Purification with Grim Reefer® Free DNA Removal



Kit Components

Item P/N	Item Name	Qty Provided
420145	Grim Reefer® Free DNA Removal Enzyme and Buffer	100 Reactions
	Grim Reefer® Enzyme	1 Bottle (2.5 mL)
	Grim Reefer® Buffer	1 Bottle (12.75 mL)
420144	Grim Reefer® Control	1 Tube (50 µL)
420143	Grim Reefer® Assay	1 Tube (200 µL)
420001	SenSATIVAx Flower & Leaf DNA Purification Kit	200 Extractions
	MGC Lysis Buffer	1 Bottle (12 mL)
	MGC Binding Buffer	1 Bottle (49 mL)
	MGC Elution Buffer	1 Bottle (12 mL)
420005	SenSATIVAx MIP DNA Purification Kit 1 gram	200 extractions
	SenSATIVAx MIP Solution A	2 Bottles (700 mL/btl)
	SenSATIVAx MIP Solution B	1 Bottle (25 mL)
	MGC Binding Buffer	1 Bottle (49 mL)
	MGC Elution Buffer	1 Bottle (12 mL)
420326	PathoSEEK® SCCG Control	1 tube (60 uL)

DNA Extraction for Flower Samples

1. Prepare the Grim Reefer (GR) reagents:
 - a. Allow a 1.5 mL tube rack to come to temperature in a 37 °C incubator
 - b. Thaw the GR Buffer and vortex to mix.
 - c. Thaw GR Control and make a 1:10,000 dilution (fresh each day):
 - i. Ensure GR Control is completely thawed and vortex to mix. Quick spin tube.
 - ii. Make a 1:100 dilution (1 uL positive control + 99 uL water. Mix well). Spin down contents of tube after vortex
 - iii. Make a second 1:100 dilution (1 uL of 1:100 dilution + 99 uL water. Mix well) - Spin down contents of tube after vortex. This is your 1:10,000 dilution.

2. After enrichment, homogenize the sample. Then, aspirate 1 mL from the side of the filter bag (free of plant debris) and dispense into a fresh 1.5 mL tube.
3. Add 112 μ L of GR Buffer to all sample tubes and mix well by vortexing (briefly spin to remove liquid from cap).
4. Add 20 μ L of GR Enzyme and mix well by vortexing (briefly spin to remove liquid from cap).
5. After the addition of the GR Enzyme, immediately incubate the tubes at 37 °C for 10 minutes. At the end of the 10-minute incubation, immediately proceed to the next step.
6. Add 50 μ L of MGC Lysis buffer to the sample.
7. Vortex for at least 10 seconds and incubate on the bench for 2 minutes.
8. Transfer 10 μ L of the diluted GR Control (1:10,000) and mix by vortexing.

NOTE: The GR Control is used to show the GR enzyme was completely deactivated during the lysis step.

9. Spin all samples for 1-3 minutes in a high-speed or bench-top mini centrifuge.

Note: The supernatant should be translucent at this point. If the sample is still opaque (cloudy), spin for longer. This is important for removing cellular debris. See Figure 4.

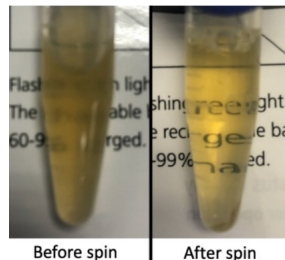


Figure 4: Example of translucent lysate after spinning.

10. Remove the 200 μ L of supernatant from the 1.5 mL tube containing the centrifuged sample, being careful not to disturb the pellet at the bottom of the tube. Place the 200 μ L in a 96-well extraction plate labeled with “Extraction Plate Day1 [date]”

Note: Pellet size will vary depending on trichome density.

11. Vortex MGC Binding Buffer thoroughly before use, and be sure the magnetic particles are completely re-suspended in the buffer for at least 30 seconds.
12. Add 200 μ L of MGC Binding Buffer to each sample, and pipette tip mix 15 times.

Note: Pipette gently when tip mixing to avoid adding too many bubbles. This is extremely important to prevent contamination of neighboring wells.

13. Incubate the plate on the bench for at least 5 minutes.

14. Place the extraction plate onto the 96-well magnet plate for at least 5 minutes.
15. After the 5-minute incubation, remove as much of the supernatant as possible (approximately 400 μ L). Be careful not to disturb or aspirate the beads. See Figure 5.
 - a. Add 400 μ L of 70% ethanol (EtOH) with the extraction plate still on the magnet plate.
 - b. Wait at least 30 seconds before removing all of the EtOH.

Note: Place the pipette tip at the bottom center of the well to remove all liquid.

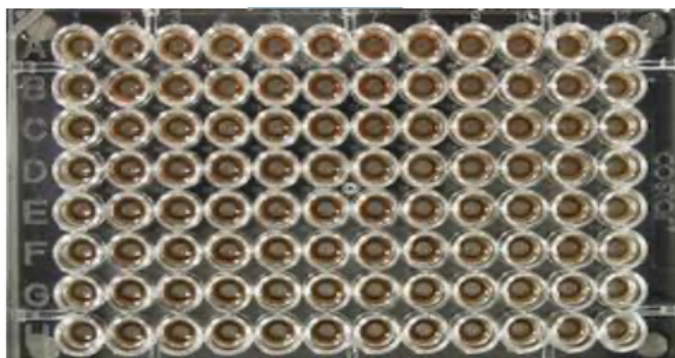


Figure 5: Extraction plate during wash step on magnetic plate.

16. Repeat 400 μ L 70% EtOH wash with the extraction plate still on the magnet plate. Wait at least 30 seconds before removing all of the EtOH.

Note: If EtOH still remains in the wells, go back in with a smaller pipette tip to remove the excess. Leftover EtOH can inhibit qPCR efficiency.
17. After all the EtOH has been removed, let the beads dry at room temperature. Allow the beads to dry for up to 15 minutes. Necessary drying time will vary based on complete removal of the second ethanol wash, as well as the lab environment. Visually inspect beads for residual ethanol before the elution step.

Note: It is important to NOT allow the beads to dry for an extended period of time. Over-drying can cause a reduction in DNA yield.
18. Remove the extraction plate from the magnet plate and add 50 μ L of MGC Elution Buffer.
 - a. Tip mix approximately 15 times or until the beads are completely re-suspended.

Note: The resuspensions may appear varied in their appearance, but the result will be the same. See Figure 6.
 - b. Incubate the plate for at least 1 minute on the bench before returning the plate to the magnetic plate.

- c. Let the plate sit on the magnet for at least 1 minute before transferring the eluent to a new extraction plate labeled with “Final Extract [date]”.

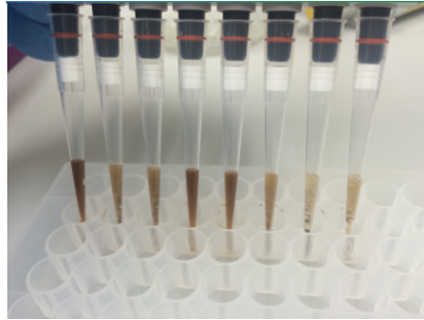


Figure 6: Multichannel pipette tips showing magnetic beads resuspended in elution buffer.

- d. Seal the plate with the adhesive seal, making sure to completely seal the plate wells using a pen or flat object to slide back and forth along the seal. Store at -20°C until ready to perform the qPCR protocol.

DNA Extraction for MIP Samples

1. Remove samples from the incubator and vortex well to thoroughly mix.
2. Transfer 1.2 mL of the mixture to a 15 mL tube.
3. Add 112 μ L of Grim Reefer Buffer.
4. Add 24 μ L of Grim Reefer enzyme and vortex to mix well.
5. Incubate at 37°C for 10 minutes.
6. Remove samples from the incubator and add 2.3 mL of MIP Solution A.
7. Vortex and incubate at room temperature for 5 minutes.
8. Vortex and transfer 1 mL to 1.5 mL Eppendorf Tube.
9. Add 10 μ L of a fresh 1:5K dilution of SCCG internal cannabis control and vortex to mix.
10. Centrifuge at 14,000 - 19,300 x RCF for 10 minutes.
11. Transfer 600 μ L from the middle layer without disturbing the pellet at the bottom and put into a fresh 1.5 mL Eppendorf Tube.
12. Add 600 μ L Chloroform and vortex vigorously until the solution is completely white.

Note: This may require longer vortexing for some matrices.

13. Centrifuge sample tube at approximately 14,000 - 19,300 x RCF for 5 minutes.
14. Pipette 100 μ L from the upper phase of the sample to a well of the extraction plate.
15. Add 100 μ L of MGC MIP Solution B to the sample, pipette tip mix three times.
16. Vortex MGC Binding Buffer thoroughly before use, be sure that the magnetic particles are completely re-suspended in the buffer for at least 30 seconds.
17. Add 200 μ L of MGC Binding Buffer to each sample, and pipette tip mix 15 times.

Note: Pipette gently when tip-mixing to avoid adding too many bubbles. This is extremely important to prevent contamination of neighboring wells.

18. Incubate the plate on the bench for at least 5 minutes.
19. Place the extraction plate onto the 96-well magnet plate for at least 5 minutes.
20. After the 5-minute incubation, remove as much of the supernatant as possible (approximately 400 μ L). Be careful not to disturb or aspirate the beads. See Figure 7.
 - a. Add 400 μ L of 70% ethanol (EtOH) with the extraction plate still on the magnet plate.
 - b. Wait at least 30 seconds and remove all the EtOH.

Note: Place the pipette tip at the bottom center of the well to remove all liquid.

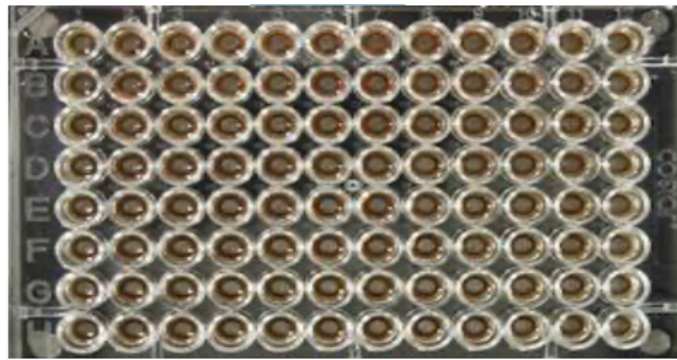


Figure 7: Extraction plate during wash step on magnetic plate.

21. Repeat 400 μ L 70% EtOH wash with the extraction plate still on the magnet plate. Wait at least 30 seconds and remove all the EtOH.

Note: If EtOH still remains in the wells, go back in with a smaller pipette tip to remove the excess. Leftover EtOH can inhibit qPCR efficiency.

22. After all the EtOH has been removed, let the beads dry at room temperature. Allow the beads to dry for up to 15 minutes. Necessary drying time will vary based on complete removal of the

second ethanol wash, as well as lab environment. Visually inspect beads for residual ethanol before the elution step.

Note: It is important to NOT allow the beads to dry for an extended period of time.

Over-drying can cause a reduction in DNA yield.

23. Remove the extraction plate from the magnet plate and add 50 μ L of MGC Elution Buffer.

a. Tip mix approximately 15 times or until the beads are completely re-suspended.

Note: The resuspensions may appear varied in their appearance, but the result will be the same. See Figure 6.

b. Incubate the plate for at least 1 minute on the bench, before returning the plate to the magnetic plate.

c. Let the plate sit on the magnet for at least 1 minute before transferring the eluent to a new extraction plate labeled with “Final Extract [date]”.

Note: It is not necessary to include the Grim Reefer Assay in the master mix for MIPs. In this case, the detection of SCCG positive control will indicate the lysis buffer deactivated the GR enzyme.

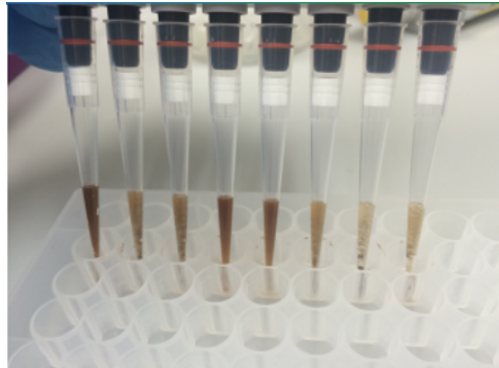


Figure 6: Multichannel pipette tips showing magnetic beads resuspended in elution buffer.

Real-Time Quantitative PCR (qPCR) Setup Protocol

1. Remove water, reaction buffer, and detection assays from the -20 °C freezer. Allow tubes to thaw at room temperature. Once thawed, immediately place tubes on ice.
2. Leave qPCR Master Mix v3 in the -20 °C freezer until ready to use or place it directly on ice.
3. Invert or vortex and spin-down the reagents.

- a. Detection Assay tubes, reaction buffer, positive controls, and water – Vortex quickly followed by a pulse spin-down in a microcentrifuge.
 - b. qPCR Master Mix v3 - Invert the tube 5 times (do not vortex), followed by a pulse spin-down in a microcentrifuge.
 - c. Return all reagents to the ice.
- Note: Do not vortex the qPCR Master Mix at any point during the protocol.*
4. Make a separate master mix in a 1.5 mL tube for each assay type being run. All detection assays contain the internal control probe and the probe for the microbial targets. Label each tube with [Assay Name] MM. Always prepare enough master mix for 1 or 2 additional reactions over the total number of tests to account for pipetting and dead volumes.
- Note: It is best to add the largest volume reagent first, in this case water.*

Table 1: PCR Reagent Volumes with Grim Reefer Assay

To detect the GR positive control spiked into flower samples during the DNA extraction using Grim Reefer, 0.5 µL of GR Assay is added per reaction. To keep the master mix identical, use GR Assay mix for all samples where Grim Reefer was used.

Reagents	1 Reaction	24 reactions (plus 1 excess rxn)	48 reactions (plus 2 excess rxn)
qPCR Master Mix	3.75 µL	93.75 µL	187.5 µL
Detection Assay	1 µL	25 µL	50 µL
Grim Reefer® Assay Mix	0.5 µL	12.5 µL	25 µL
Reaction Buffer	0.8 µL	20 µl	40 µl
Water	7.7 µL	192.5 µL	385 µL
Total MM	13.75 µL	343.75 µL	687.5 µL

Table 2: PCR Reagent volumes without Grim Reefer[®] Assay For MIP samples where Grim Reefer[®] Control was not added.

Reagents	1 Reaction	24 Reactions (Plus 1 excess rxn)	48 Reactions (Plus 2 excess rxn)
qPCR Master Mix	3.75 µL	93.75 µL	187.5 µL
Detection Assay	1 µL	25 µL	50 µL
Reaction Buffer	0.8 µL	20 µL	40 µL
Water	8.2 µL	205 µL	410 µL
Total MM	13.75 µL	343.75 µL	687.5 µL

5. Once combined, gently tip mix or invert the tube 5 times to combine the assay master mix.
 - a. Pulse spin-down tube in microcentrifuge.
 - b. Place qPCR Master Mix tubes on ice until used.
 - c. For the positive control, make a 1:10 dilution
 - i. Add 1 µL of Positive Control to 9 µL nuclease free water (found in the kit), vortex to mix well and spin down the tube.
 - ii. For the negative control, use water (found in the kit)

Note: It is best to add the largest volume reagent first. In this case, add the 9 µL water first, then the 1 µL of positive control. Pipette mix or vortex to ensure the control DNA is in solution.
6. Place the Extraction Plate on the magnet. This is to ensure no magnetic beads are transferred into the qPCR reactions if there are some left over from the extraction elution process.
7. Use a 96-well optical qPCR plate and label the plate “qPCR Plate_ [date]”.
8. Carefully remove the seal from the Extraction Plate. If frozen, let the DNA thaw completely, spin the plate to avoid cross-contamination between samples, and pipette tip mix the DNA.
9. Transfer 5 µL of each sample into the corresponding well on the qPCR plate. Keep the extraction plate on the magnet when aspirating the 5 µL.
 - a. Add 5 µL of the diluted Positive Control to the corresponding positive control well.
 - b. Add 5 µL of water to the corresponding negative control well.

Note: ALWAYS use a fresh tip for every liquid transfer into the qPCR plate

10. Add 13.75 µL Master Mix to each corresponding sample well, positive control well, and negative control well in the qPCR plate. Gently tip mix a few times after each addition of qPCR master mix. Be careful not to introduce bubbles during this mix.
11. Seal the plate with strip caps or an adhesive seal.
12. Spin down for at least 1 minute in plate microcentrifuge to bring well contents to the bottom of wells and help to get rid of reaction bubbles.

Note: Check for bubbles at the bottom of the wells (minimal bubbles on the surface of the liquid is acceptable). If bubbles remain in the bottom of the wells, spin-down for another minute.

13. For the Agilent Aria: If using an adhesive seal, place the reusable compression pad (gray side down) on the plate directly, lining up the holes in the pad with the holes in the plate.
14. Place the sealed plate onto the PCR instrument, positioning the A1 well in the top left corner.
15. Follow the software-specific instructions to initiate the run.

PathoSEEK® Data Analysis Quick Reference Table

PathoSEEK® Target	Cq Value	Fluor	Negative Control (Cq)	CFU threshold (CFU/g)
Internal Control*	≤35	HEX	*Internal control verifies the presence or absence of plant DNA and verifies successful inactivation of Grim Reefer Enzyme for MIP samples. ** Grim Reefer Pos control verifies successful inactivation of Grim Reefer Enzyme for flower samples.	
Grim Reefer Pos Control**	≤35	Cy5		

Results

For detailed data analysis refer to the appropriate User Guide. When using Grim Reefer® in conjunction with the bacterial detection assays, the following guidance should also be observed:

Amplification of Cy5 should be observed with a Cq of ≤35 in all sample reactions in which the Grim Reefer control was spiked in after lysis buffer addition and curves should be visually assessed for appropriate amplification.

Troubleshooting Guide for Grim Reefer®

Symptom	Reason	Solution
No Amplification of Grim Reefer Control in Flower or Internal Control in MIPs	Grim Reefer Control dilutions not freshly prepared	Make a fresh GR control dilution. Repeat SenSATIVax® and PathoSEEK® by following the protocol.
	Lysis Buffer was not added/insufficient vortexing after lysis buffer addition	GR Enzyme was not deactivated and GR Control DNA Was degraded
	Mix up in qPCR Reaction Setup	Repeat the qPCR by following the protocol.
	Missing Cy5 Fluorophore on plate set up	Check plate setup in qPCR platform data file and ensure the Cy5 fluorophore was chosen for the wells that include the GR Control spike
	qPCR inhibition	Dilute Elution 1:10 with nuclease free water and re-perform qPCR
Total run failure	Excessive vortex of the qPCR Master Mix	Repeat the qPCR by following the protocol.

REVISION HISTORY

Version	Date	Description
v1	March 2025	Creation of supplemental guide for use of Grim Reefer® Free DNA Removal with PathoSEEK® Bacterial Detection Assays with SenSATIVax® Purification.

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.

This test has not been validated on remediated (irradiated, ozone treated, acid treated, hydrogen peroxide treated, etc.) samples. Samples that have undergone remediation may cause discordant results between plating methods and PathoSEEK® methods. When remediated samples produce a result above the action limit on qPCR, we recommend confirming viability with an approved plating method.

[A third party verification study was performed to support the use of Grim Reefer® with MIPs and can be found here.](#)

Important Note: The PathoSEEK® Salmonella & STEC Multiplex Assay with SenSATIVAx® DNA Purification AOAC PTM validation was conducted without using MGC's Grim Reefer® Free DNA Removal Kit.

The results may vary based on laboratory conditions. Altitude and humidity are among factors known to affect the growth of bacterial and fungal species.

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