

DNA ISOLATION INSTRUCTIONS

The following sampling instructions are used for the field isolation of DNA from a Bio-Flo™ filtered sample using the Bio-Extract™ field kit, supplied by Microbial Insights, Inc.

Be sure to keep the Bio-Flo™ filter closed until DNA isolation is performed. Always wear latex gloves (or similar) when performing this protocol. Please ensure that all tools and instruments are returned to Microbial Insights, Inc.

Notes: DNA can be isolated from multiple filters simultaneously, provided there is adequate room for shaking the samples at the same time.

Clean your work area between each DNA isolation sample batch to avoid contamination between samples.

Procedure:

- 1) Label the filters with their respective sample IDs.
- 2) Uncap both ends of the filter and set the caps aside for use later. Find and open one of the 3 mL syringes included in the kit. Pull air into the 3 mL syringe and connect it to the filter via the luer-lok connection. Push the air out of the syringe so as to push any remaining water out of the filter. Disconnect the syringe and pull in more air before repeating again. Repeat several times, ensuring that as much water as possible is pushed out of the filter. Re-cap the red outlet cap.
- 3) Invert the closed Solution A syringe several times to ensure it is well mixed. Remove the luer-lok syringe cap.
- 4) Connect the Solution A syringe to the Bio-Flo filter inlet via luer-lok and slowly add all of the Solution A by depressing the plunger. Pushing too fast can cause back-pressure to form so be sure to lower the plunger slowly. If filter clogging is preventing the addition of Solution A, remove the red outlet cap of the filter and try again. Re-cap the filter.
- 5) Vigorously hand-shake the closed filter for 5 minutes.
- 6) Invert the closed Solution B syringe several times to ensure it is well mixed. Remove the luer-lok cap on the syringe.
- 7) Remove the Bio-Flo filter inlet cap. Connect the Solution B syringe to the Bio-Flo filter inlet via luer-lok and slowly add all of the Solution B by depressing the plunger. Pushing too fast can cause back-pressure to form so be sure to lower the plunger slowly. Re-cap the filter inlet cap.
- 8) Vigorously hand-shake the closed filter for 5 minutes.
- 9) Remove the inlet cap.
- 10) With the filter inverted, push 1 ml of air into the filter using a 3 ml syringe attached to the Bio-Flo™ filter unit and then pull back on the plunger to remove as much of the lysate (liquid) as possible. Note: If lysate is particularly viscous, it may be necessary to push and pull the plunger several times to remove the solution from the filter.
- 11) Add the lysate to a 5ml bead tube. Cap the tube and parafilm the top.
- 12) Vigorously hand-shake the tube for 5 minutes. Sit tube upright while continuing on with the steps.
- 13) While avoiding contamination of the foil top, place the Bio-Extract™ sample prep cartridge onto a flat surface.

- 14) Remove the 1 mL syringe from its packaging and screw the luer-lockable column onto the end of the syringe. Use the pointed tip of the column to poke two holes into the first section of the Bio-Extract™ sample prep cartridge.
- 15) Using a plastic volumetric pipette, transfer 1 mL of lysate (liquid only, avoid transferring beads as much as possible) from the bead tube into the newly punched hole of the first Bio-Extract™ sample prep cartridge section. For best results, slowly add the lysate at a slight angle when pipetting into the hole in the foil.
- 16) Place the syringe with the attached Sample Prep Column back into the red section of the cartridge and draw the fluid all the way up the syringe and pump all the way back out. Repeat for a total of 10 pumps.
- 17) Push all of the fluid back into the red section of the cartridge prior to the next step. Do not transfer any liquid from one section of the cartridge to the next. This applies to each remaining step of the sample DNA isolation protocol.
- 18) Move the 1 mL syringe with the attached Sample Prep Column into the red-orange section of the Sample Prep Cartridge and pierce through the foil. Remember to pierce 2 holes per section of the cartridge to minimize liquid splatter, except during the Air-Dry step. Draw the fluid all the way up the syringe and pump all the way back out. Repeat once for a total of 2 pumps assuring that no buffer remains in the syringe before beginning the next step.
- 19) Move the 1 mL syringe with the attached Sample Prep Column to the orange section of the Sample Prep Cartridge and pierce two holes through the foil. Draw the fluid all the way up the syringe and pump all the way back out once assuring that no buffer remains in the syringe before beginning the next step.
- 20) Move the 1 mL syringe with the attached Sample Prep Column to the yellow section of the Sample Prep Cartridge and pierce twice through the foil. Draw the fluid all the way up the syringe and pump all the way back out once assuring that no buffer remains in the syringe before beginning the next step.
- 21) Move the 1 mL syringe with the attached Sample Prep Column to the blue air-dry section of the Sample Prep Cartridge and pierce through the foil only once. Draw air up through the syringe and quickly pump back out. Repeat pumping vigorously 20 or more times until the Sample Prep Column appears dry and does not spray fluid droplets.
- 22) Move the 1 mL syringe with the attached Sample Prep Column to the green section of the Sample Prep Cartridge and pierce through the foil twice. Elute by drawing the fluid all the way up through the syringe and slowly pump back out for a total of 5 pumps.
- 23) That's it! Using the 1 mL syringe and Sample Prep Column, transfer all of the solution from the green section of the sample prep cartridge into a well-labelled 1 mL microtube and store at ≤ 4 °C until use.
- 24) If you are using the extracts for Field-Quant™ analysis, move directly to the Field-Quant™ protocol. If you are shipping the extracts for laboratory analysis, follow the steps below.

To Submit Sample:

1. Parafilm the top of each microtube and place it into its respective falcon tube that previously held the Bio-Flo™ filter kit, ensuring that the falcon tube is labelled with the sample name, date and filtered volume.
2. Be sure to fill out the Chain of Custody (COC) form correctly and accurately and ship it along with the samples. A COC form is required for QA/QC purposes.
3. Once the falcon tubes have been correctly labeled, place them in the designated cooler.
4. Be sure to fill the remaining space in the cooler with ice.

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5. All paperwork to be sent with the samples should be placed within a waterproof pouch or Ziploc bag and placed on top of the samples or affixed to the inside lid of the cooler.
6. Please ensure that all tools used for sample prep, even those unused, are returned to Microbial Insights along with the samples.
7. Seal the cooler lid with a strong packaging tape.

SHIPPING INSTRUCTIONS

Packaging Samples:

1. Samples should be shipped in a cooler with ice or blue ice for next day delivery. If regular ice is used, the ice should be double bagged.
2. A chain of custody form must be included with each shipment of samples. Access our chain of custody at www.microbe.com.
3. Upon receipt at the laboratory, Microbial Insights will ensure that the samples are preserved for future analyses and long-term storage.

Shipment for Weekday Delivery:

Samples for weekday delivery should be shipped to:

Sample Custodian
Microbial Insights, Inc.
10515 Research Drive
Knoxville, TN 37932
(865) 573-8188

Shipment for Saturday Delivery:

Coolers to be delivered on Saturday must be sent to our **FedEx Drop Location**. To ensure proper handling the following steps must be taken:

1. FedEx shipping label should be marked under (6) Special Handling, check Hold Saturday.
2. The cooler must be taped with FedEx SATURDAY tape.
3. The shipping label must be filled out with the Drop Location address below. Our laboratory name must be on the address label.
4. You MUST **notify by email** customerservice@microbe.com with the tracking number of the package on Friday (prior to 4pm Eastern Time) to arrange for Saturday pickup. Please make sure you write "Saturday Delivery" in the subject line of the message. **Without proper labeling and the tracking number, there is no guarantee that the samples will be collected.**

Samples for **Saturday delivery** should be shipped to:

Microbial Insights, Inc.
FedEx Drop Location
10601 Murdock Drive
Knoxville, TN 37932
(865) 300-8053