



## Proper Sampling of Soil Using MicroTally® MicroBootie™ Samplers



### Introduction

The **MicroTally® MicroBootie™ Samplers** (Catalog Number: MT-MB100) are specifically designed for footprint sampling of soil in agriculture field and other surface substrates that are walked upon, but this protocol focuses on the needs of the produce production field. The MicroBootie sampler is best used with the provided liner to prevent sample contamination from the shoe(s) to the sampler. After sample collection, the MicroBootie sampler facilitates downstream microbiological analysis for pathogen detection and indicator testing, providing an efficient alternative to direct soil testing. In some instances, having two highly matched samples, one from each foot, can facilitate projects where two sample processing protocols are required such as when one sample will be prepared with a non-cultured protocol and the other will be incubated for enrichment. Accurate and reliable sampling results depend on strict adherence to standard sampling protocols. This user manual focuses on the proper sampling procedures for soil using MicroTally® MicroBootie™ samplers. For additional instructions and applications for the MicroBootie sampler, please contact FREMONTA Corp or visit [www.MicroTally.com](http://www.MicroTally.com). Training videos can also be found on FREMONTA's YouTube channel.

### Important Notices

- **Sterility:** MicroBootie™ Samplers are sterile, individually packaged in resealable bags, and provided with sterile liners.
- **Sample quantity:** A single sampler is typically sufficient; use two if laboratory protocols require multiple processing streams.
- **Pre-moistening:** For soil sampling, particularly in dry conditions, pre-moisten the sampler with 25 mL of sterile 10% propylene glycol to facilitate pathogen transfer.
- **Timing:** Sampling shortly after tilling is recommended to mitigate the effects of solarization on microbial recovery.
- **Technique:** Walk normally to allow body weight to facilitate transfer. Avoid scuffing or dragging feet to prevent mechanical abrasion of the sampler.
- **Distance:** Sampling paths typically range from 20 m to 200 m. Reduce the distance on rough surfaces (e.g., dry soil) or with heavier users to prevent excessive wear.
- **Post-sampling:** Return the used bootie to the resealable bag for transport and discard the sterile liner.

### Preparation

Prior to entering the sampling area, prepare the MicroBootie™ samplers as follows:

1. **Sanitization:** Don gloves and sanitize them according to standard operating procedures.
2. **Bag Preparation:** In a designated area, remove perforated top from the MicroBootie™ sample bag. Apply barcodes to bags at this time if applicable.
3. **Hydration:** Add 25 mL of sterile 10% propylene glycol solution to each bag. Gently massage the bag to ensure the sampler is evenly moistened.
4. **Sealing:** Fold the bag opening and secure it using the integrated side tabs.
5. **Storage:** Samplers may be prepared in advance for daily use; store refrigerated until needed.
6. **Staging:** Consolidate all supplies (liners, moistened samplers, and sanitizer) in a transport tote or tool belt. Ensure all materials are accounted for post-sampling to maintain GAP/GMP compliance.

### Sampling Procedure

1. Assemble the appropriate sampling supplies.
2. Plan sampling path based on the testing objective. This may involve wearing the sampler while performing



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other duties. Scan the barcode if appropriate.

- Sanitize gloves appropriately using an alcohol-based sanitizer **without** quaternary ammonium compounds (e.g., Alpet D2 **Quat-Free**; Best Sanitizers, Penn Valley, CA, 70% Isopropyl alcohol is another alternative) to apply and remove samplers.
- Ensure there is no excess sanitizer on the gloves before handling the MicroBootie™ sampler.
- Wear a new plastic liner for each sampler to assure sample independence.
- After collecting the samples by walking the planned distance, remove and fold each MicroBootie™ sampler into quarters, and then fold it in half once more. Return the sampler to the sample bag, ensuring positioning the MicroTally® MicroBootie™ sampler as close to the bottom of the sample bag as possible. Securely close and fold over the bag using the sealing strip located at the top. Remember to label the bag in accordance with company procedures.
- Sampling details:
  - Rough freshly disced soil: This soil is often very abrasive, avoid breaking the liner and contaminating the sample.
  - Furrows: It is generally desirable to sample as close to plants as possible to better reflect conditions under the canopy.
  - Muddy soil: The goal is to sample the soil and not the water. Water should be squeezed out or avoided. Consider what is being sampled.
- Repeat the steps as necessary for the other MicroBootie™ samples to be collected.
- Please note that each sampling path will require a new MicroBootie™ sampler and liner to avoid cross-contamination. It is crucial to change gloves and re-sanitize after each sampling event to maintain proper hygiene and prevent any potential sample contamination.
- Send the samples to the laboratory following standard company procedures.

### Sample Testing

- For presence/absence pathogen testing, enrich each MicroTally® MicroBootie™ sampler by adding 200 mL of the appropriate nutrient broth. Stomach to homogenize)the sample for at least 30 seconds, ensuring the sampler is fully submerged at the bottom of the bag.
- For indicator enumeration and pathogen testing, remove aliquots as needed. Incubate the remaining sample following the standard procedures of your pathogen detection test platform, including recommended incubation times and temperatures.
- For microbial enumeration-only testing, you may extract microorganisms using a smaller liquid volume to concentrate the target organisms.

### Technical Support

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