

# BIOME-Preserve

## BIOME-Preserve: A collection kit for culturing the microbiome

BIOME-Preserve is a collection kit that preserves live microbiota samples for growth and isolation of anaerobic and facultative anaerobic microorganisms. The product effectively preserves microorganisms under anaerobic conditions at room temperature for up to 120 hours without substantial loss of viability. Samples may also be directly frozen in BIOME-Preserve for cryogenic storage.

## BIOME-Preserve enhances recovery of live microbiota in a convenient and cost-effective manner

The collection and culture of organisms from complex biological samples, such as stool, is difficult. Obligate anaerobic organisms are killed in the presence of atmospheric oxygen. Acidic and other metabolic byproducts also can kill microorganisms in the sample if growth continues after sample collection. The growth and proliferation of some fast-growing organisms in a sample prevent the recovery of low abundance, fastidious, or slow growing organisms.

BIOME-Preserve was developed in response to a need for a practical, low cost, and user-friendly system to collect, transport, and preserve microbiota samples to culture microorganisms within the sample. Anaerobe System's proprietary and patent pending collection device preserves viability of microorganisms temporarily stored in the kit at room temperature for up to 5 days. The same collection medium and container can also be frozen to preserve organisms for future culture recovery.

## BIOME-Preserve Product & Usage Information

**Collection Device:** Stool collection tube with an integrated 1ml collection scoop attached to the cap. Tube is clear polypropylene plastic filled with 12mL of BIOME-Preserve liquid medium. Custom selected container configurations available on request.

**Preservative:** Liquid medium, pre-reduced & anaerobically dispensed into tubes. Medium is non-nutritive phosphate buffer containing mineral salts, oxygen scavengers, antioxidants, & cryopreservants.

**Storage & Shelf Life:** Product can be stored at room temperature or refrigerated. Avoid freezing prior to use. Short term exposure to higher temperatures, such as during shipping/mailing, will not affect product performance. BIOME-Preserve should be used within 6 months from date of manufacture. Microbiota samples, when collected prior to product expiration, may be preserved and stored at -80°C for extended periods beyond expiration. Collection tubes are individually packaged in foil pouches under anaerobic conditions.

### Sample Collection:

- *Important product note:* Do not remove the tube from the foil package until the time of sample collection.
- Collect the microbiota sample in a suitable container or toilet accessory. Do not collect from the toilet bowl directly.
- Remove the BIOME-Preserve tube from the outer foil packaging and remove the cap from the BIOME-Preserve tube.
- Collect 1 scoop of stool or microbiota sample, place the scoop in the BIOME-Preserve tube, and tighten the cap. The tube should not stay opened for longer than 1 minutes.
- Shake the sealed tube vigorously for 10 seconds to homogenize the sample as best as possible.

**Sample Transport & Storage:** For optimal recovery, it is recommended to transport and process samples within 96hrs of sample collection. Longer transport times are possible, up to 120 hours, with a lower rate of recovery. Samples are intended to be stored and transported at room temperature in BIOME-Preserve. Refrigerating a sample is not necessary but may help slow potential growth/metabolism of organisms. Temporary freezing for the purposes of shipment is not recommended unless samples will remain frozen until processing, and users should avoid multiple freeze thaw cycles. Samples may be frozen at -80C for long term storage. Check with local/state/country regulations for labeling and packaging requirements for shipping stool samples.

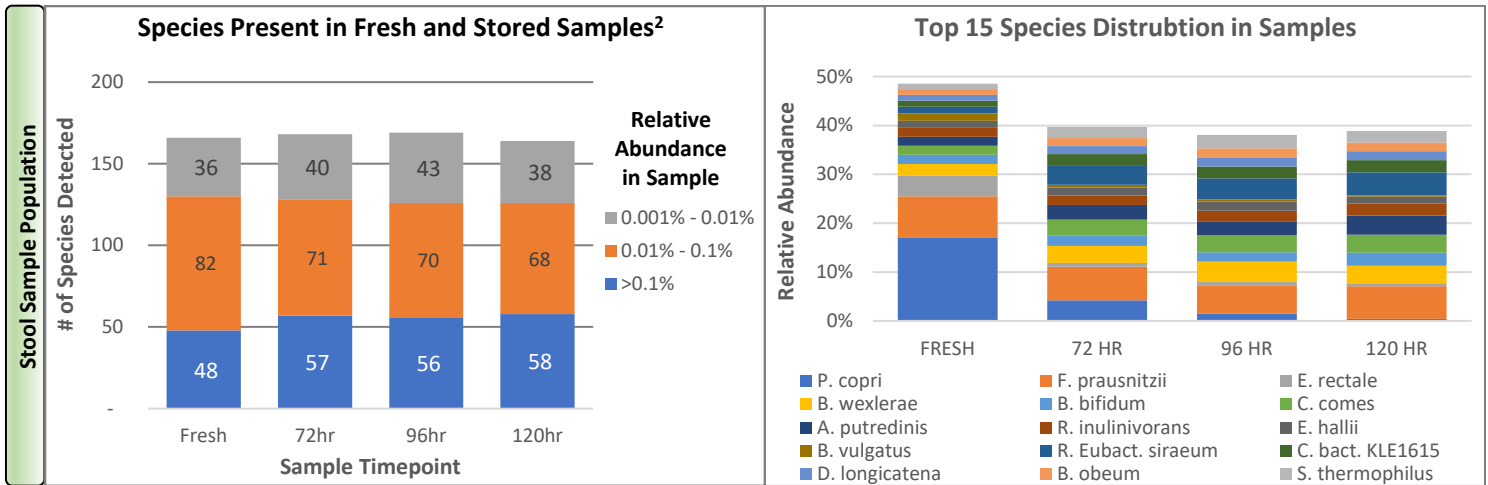


## Case Study: Culture Recovery of Stool Sample Held for Various Time

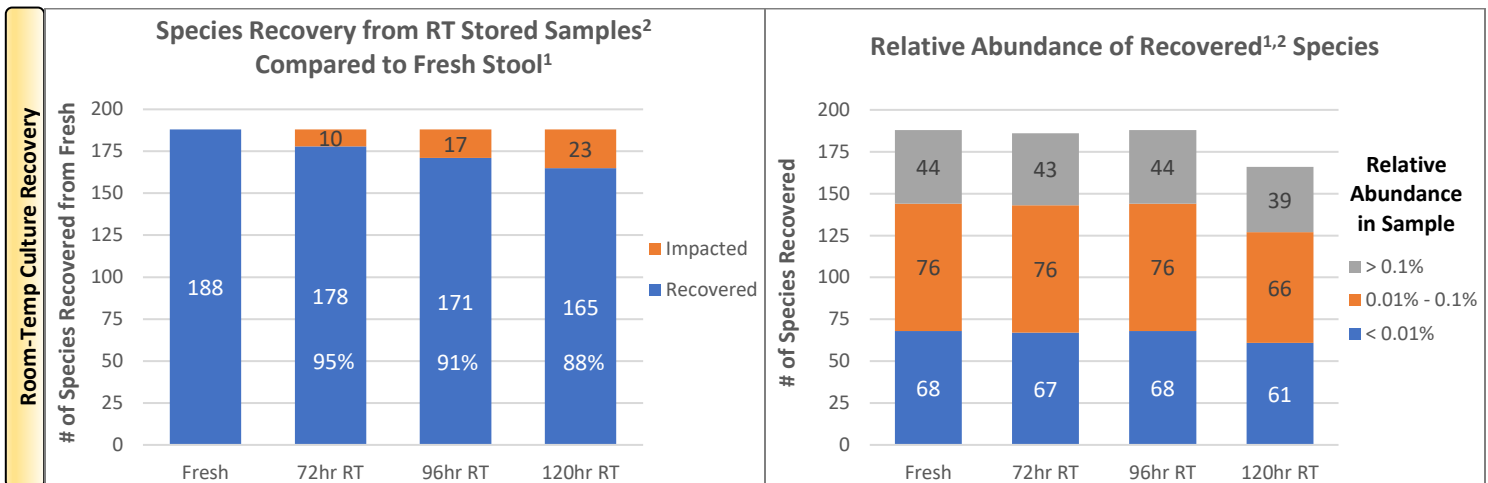
Stool from a single donor was homogenized, added to BIOME-Preserve in air, and stored for various time points. A subset of the tubes was frozen at -80C for 7 days following storage. All conditions were set up in triplicate. Fresh homogenized stool, stool stored in BIOME-Preserve, and stool stored in BIOME-Preserve then frozen were anaerobically cultured on a variety of solid agar media to compare species recovery. Bacterial growth was harvested and processed for shallow whole genome shotgun sequencing (avg 4 million reads per sample). OTUs identified at the species level following a filtering protocol were analyzed. Species with a relative abundance in harvested bacterial materials >0.01% were considered successfully recovered.

# BIOME-Preserve

## Microbial Diversity Maintained in Samples Held in BIOME-Preserve

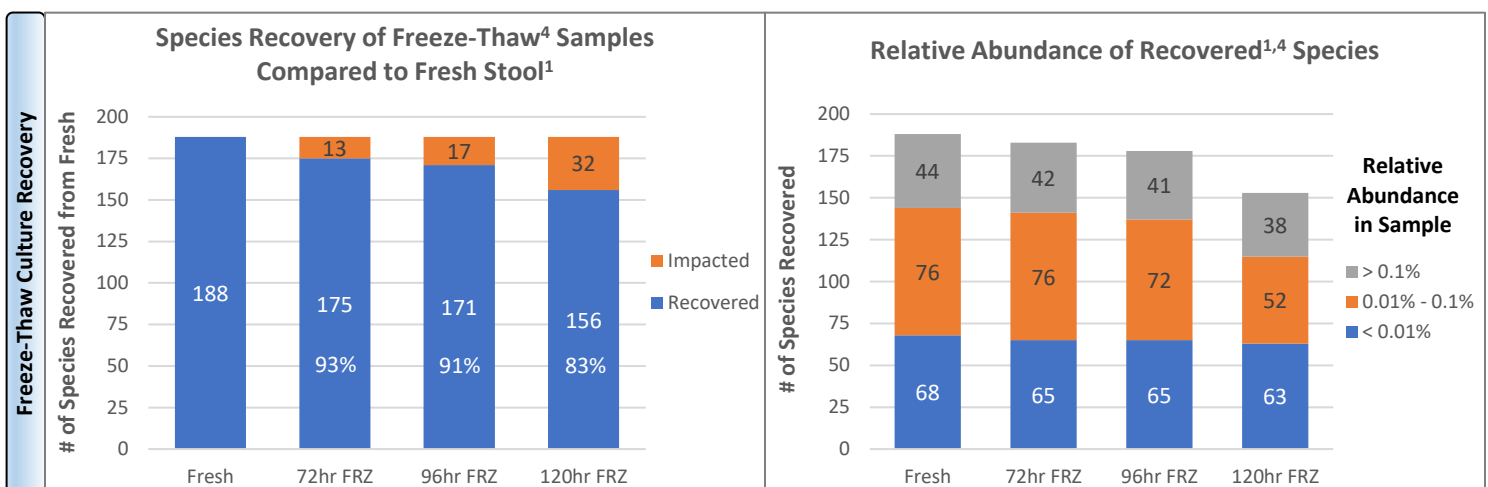


## BIOME-Preserve Maintains Culturable Diversity of Room Temperature Held Samples



Impacted: Species with a > 10-fold (1-factor) drop in relative abundance in harvested cultured cells as compared to relative abundance in fresh stool culture

## BIOME-Preserve Maintains Culturable Diversity of Samples Following -80°C Storage<sup>4</sup>



<sup>1</sup> Fresh stool sample homogenized and processed for anaerobic culture as described in (3).

<sup>2</sup> 1g homogenized stool from (1) added to BIOME-Preserve in air. Stored for specified time period at room temp prior to anaerobic culture as described in (3)

<sup>3</sup> Samples spread on a variety agar media in triplicate, plates incubated anaerobically for 120hrs, and bacterial mat from triplicate plates combined for sequencing. Cultivated bacteria were characterized by shallow WGS & analysis. Species detected in culture were considered recovered if >0.01% relative abundance.

<sup>4</sup> Stool stored in BIOME-Preserve at RT for specified time, frozen at -80°C for 7 days, then thawed and cultured inside anerobic chamber as described in (3).