

Preparation & Shipping Procedures

For Microbiome Projects that Include Metagenomics Sequencing Services

Biological samples, particularly fecal samples, can have active microbial growth and enzyme activity, making it crucial to carefully plan how to best collect, process, store and ship samples. Following these instructions will help maintain sample quality, avoid processing delays, and ensure that the metabolomics and metagenomics data generated accurately reflect the biology of the sample.

Plan for Successful Sample Collection

- ▶ Metabolon accepts a wide variety of sample types for metabolomics analysis and 16S amplicon sequencing, and multiple fecal sample collection methods for shotgun sequencing (See the table on the next page).
- ▶ Please consult with a Metabolon representative to confirm your sample type is suitable for each analysis.
- ▶ **Regardless of collection method, please note that consistent sample handling is critical to study quality. For best results, handle all samples in a study with the same collection technique, time between collection and freezing, and the same number of freeze-thaw cycles.**
- ▶ Plan to minimize time between collection and freezing.
- ▶ For fresh frozen samples, transfer the sample amount needed into pre-labeled and pre-chilled tubes appropriate for storage at -80°C. Flash-freeze samples in liquid nitrogen and immediately store them in a -80 °C freezer. Follow vendor instructions for collection tubes like DNA Genotek.
- ▶ To ensure accurate integration of the metagenomics and metabolomics data, it is crucial to homogenize fecal samples – which can be quite variable across the initial unprocessed sample – before aliquoting the samples for their two uses. Without homogenization before aliquoting, insights from integration of metabolomics and metagenomics could be confounded and misleading.

Accepted Sample Types with Risk Levels (Preferred vs. Risky)

Note that our shotgun metagenomics sequencing is only optimized for fecal sample types

16S Amplicon Sequencing and Metabolomics can use a range of sample types.

Consult Metabolon to confirm compatibility.

		Metabolomics		Notes
Sample Description		Global Discovery Panel (GDP) Microbiome Panel (MP)	Metagenomics	
Preferred	Fecal: 2 DNA Genotek Tubes	1 DNA Genotek ME-200	1 DNA Genotek OMR-200 or OMR-205	Convenient home collection flexibility Follow collection Instructions from the vendor
	Fecal: 2+ Fresh Frozen Samples	1+ aliquots	1 aliquot	Homogenize sample before aliquoting
	Other Sample Types	GDP: broad sample types MP*: human plasma, serum, urine, fecal	Shotgun: fecal only 16S: broad sample types	Sample needs for metabolomics can be found in the Sample Submission Guide for GDP & CLP for multiple sample types.
Risky	Single Fresh-Frozen Fecal Sample	Metabolon will thaw and dilute samples in water to homogenize by vortexing before aliquoting for analyses**		Processing requires an extra freeze-thaw cycle to generate samples for each analysis
	Isolated DNA	Not suitable for metabolomics	Dependent on prep quality A260/A280 = 1.8-2.0	Standard DNA buffers – water, EB, TE (EDTA <0.1 mM), prefer fragment size > 5kB
	Single DNA Genotek ME-200 Tube	DNA Genotek ME-200 is optimized for metabolomics; using it for metagenomics risks DNA degradation & low DNA yield		DNA Genotek OMR-200 and OMR-205 are optimized for nucleic acids; using these tubes for metabolomics analysis is not acceptable.
	Lyophilized Samples	GDP: Risky MP: Not suitable	Process can bias community composition	Dry down can distort microbe community composition & metabolism

*The SCFA component of the Microbiome Panel is validated for human plasma, serum and fecal samples; samples from other animals can be accepted with some risk. For other liquids, including urine, we will use the protocol that was validated for plasma and serum.

**Metabolon has not validated the homogenization method; therefore, this processing step has associated risks.

Not accepted for shotgun metagenomics: Fecal swabs, cecal contents, any sample type with substantial host DNA (tissues), ethanol preps.

Not accepted for any analysis with Metabolon: highly degraded samples, RNeasy, formalin- or paraffin-treated, other preservatives, BSL3 or BSL4 samples.

Sample Amounts

Calculate the total sample amounts needed for a project by adding the individual amounts for metagenomics and metabolomics from the table below. Consult the [Sample Submission Guide for GDP & CLP](#) for information on additional sample types and the Biological Insights delivered with Metabolomics of Varying Sample Amounts Guide for information on how reduced sample amounts impact metabolomics data. Consult with a Metabolon representative about options if you are unable to provide these recommended amounts.

Analysis Type	Sample Type	Recommended Amount
Metagenomics – Shotgun: Fecal Samples Only 16S: Broad Sample Types	Solid Material	100 mg best (50 mg minimum)
	DNA Genotek OMR-200 or OMR-205	Full collection tube preferred**
	Extracted DNA	500 ng @ 20 ng/μL
	Other	Consult with a Metabolon Representative
Metabolomics – Global Discovery Panel Broad Sample Types	Biofluids	150 μL
	Solid Material	100 mg (feces) 30-50 mg for other tissue
	DNA Genotek ME-200	Full collection tube preferred**
Metabolomics – Global Discovery Panel + SCFA Panel Human Plasma, Serum, Urine, Feces	Plasma, Serum, Urine*	250 μL
	Fecal Solid Material	250 mg
	DNA Genotek ME-200	Full collection tube preferred** (1.5mL per panel)
Metabolomics – Microbiome Panel (incl. SCFA) Human Plasma, Serum, Urine, Feces	Plasma, Serum, Urine*	150 μL
	Fecal Solid Material	150 mg
	DNA Genotek ME-200	Full collection tube preferred**

*Note that the SCFA assay portion of the panel is only validated for plasma and serum. For other liquids, including urine, we will use the protocol that was validated for plasma and serum.

** Full collection tube contents preferred to enable best homogenization practices; 1.5 mL aliquot minimum (homogenize before aliquoting).

Sample Manifest Preparation

Your Metabolon representative will provide a manifest template for you to complete and provide both electronically and in the shipment box. It is crucial to use this manifest template (not a custom form). The manifest template will include instructions for completion and minimally requires this information:

- ▶ **Project Codes:** Metabolon will assign up to 3 Project Codes for metagenomics and metabolomics sample sets. These codes are key for sample and project tracking. Please do not ship samples without Project Codes.
- ▶ **Sample IDs:** A unique identifier for each sample must match physical label or barcode on the sample tubes.
- ▶ **Sample Type and Amounts:**
 - ▶ For liquid samples (Volume)
 - ▶ For solid samples (Weight)
 - ▶ For fecal samples or slurries (Sample volume and estimated dry weight of solid material)
- ▶ **Preparation method:** Describe how the samples were collected or preserved (e.g., flash frozen, DNA Genotek tube type or stabilization buffers, for example).
- ▶ **Samples for Metagenomics:** For projects that include metagenomics analysis, specify which samples are intended for metagenomics.
- ▶ **Special Handling Instructions,** if applicable.
- ▶ **Privacy:** Manifests **must NOT include** any personally identifying information (PHI) for human samples.

Metabolon can provide a Sample Handling kit which includes 2D-barcoded tubes. Preferred, client-supplied sample tube types (i.e. DNA Genotek, see above for details) are also acceptable. If you plan to use client-supplied barcoded tubes, please consult Metabolon in advance.

Manifest Submission & Sample Shipping

By carefully following these guidelines, you can ensure that your samples arrive at Metabolon in optimal condition, facilitating accurate and efficient processing.

- ▶ Please work with Metabolon to provide a Purchase Order (PO) and receive a Project Code before you ship your samples.
- ▶ Prior to shipping samples, email an electronic copy of the completed sample manifest in Excel format to: samplemanager@metabolon.com with the Project code in the subject line of the email.
- ▶ Print and include a hard copy of the completed manifest in the box, but outside of the Styrofoam container.

Please do not ship samples without a Project Code and Manifest

Shipping tips:

- ▶ Pack with 8-12 kg of dry ice and consider using a carrier who will top off dry ice in cases where shipping is delayed.
- ▶ Ship all samples for a project together at once. All samples for metabolomics and metagenomics need to be shipped to Metabolon at the same time.
- ▶ International shippers should work with a Metabolon Client Success Manager to ensure proper documentation is completed to reduce the risk of import delays.
- ▶ Shipping known BSL-2 level samples must be pre-approved by Metabolon and may require additional guidance. BSL-3 and above are not accepted by Metabolon.

Sample Disposition

Note that the default process is to destroy and discard leftover sample material 90 days after metabolomics data are uploaded to the portal (project completion). Please inform your Metabolon Representative if samples should be returned or require additional storage, so that the custom handling instructions can be captured in the quote and project statement of work. Note that the leftover samples provided for metagenomics sequencing analysis cannot be returned to the client if this service is not specified at the time of the contract.

Please send samples to this address:

Metabolon Sample Acceptance
617 Davis Drive, Suite 400
Morrisville, NC 27560
Phone: +1-919-572-1711

Ship domestic overnight Monday - Wednesday.

Ship international via a provider that will replenish dry ice in transit to mitigate sample impact in the event of a shipping or customs clearance delay.

Consult with your courier for preferred days to ship samples to NC.

Our receiving hours are Monday to Friday from 9AM to 4PM ET.

Consider U.S. Holidays before shipping.

Metabolomic insights: Don't get left behind

Metabolon has been at the forefront of metabolomics utilization, having executed over 15,000 projects over the last 25 years. Metabolon revolutionizes metabolomics studies with its cutting-edge technology and comprehensive analytical solutions. It empowers researchers to delve deeper into the complexities of biology, providing invaluable insights into health, disease, and beyond. With its unrivaled accuracy, efficiency, and depth of analysis, Metabolon accelerates discovery, enabling the identification of novel biomarkers, elucidation of metabolic pathways, and advancement of precision medicine. Our unique ability to provide key data and interpret them for our clients makes us a reliable collaborator as you navigate complex biological questions to drive scientific progress.

**To learn more about
how Metabolon can
help your study, please
contact us today.**

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www.metabolon.com/contact-us

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