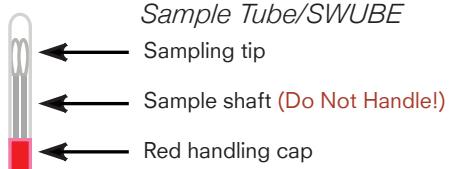




Quicklist Instructions & Checklist

Please read the entire packet before starting.
Check off each step once it has been completed.

What Your Kit Contains



Sample Tube/SWUBE

This will be used to mail your sample back. Please note that proper postage is required when sending the kit back.



Credentials Sheet

This will contain your assigned kit ID, password, and barcode number(s). **Retain this sheet for your records as your kit ID is permanent** for logging into the participant website.

1 REGISTRATION

Register your kit at <http://microbio.me/AmericanGut/> using a desktop computer or laptop.



2 VERIFY YOUR KIT

Input your emailed verification code (sent after completing Step 1) and confirm that you have received the correct barcode numbers found on your sample tubes.



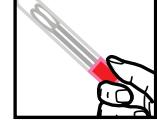
3 CREATE A PARTICIPANT PROFILE

This will take you through the required consent process, participant survey, and optional surveys, if you are adding a human sample.



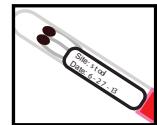
4 COLLECT SAMPLE

See Appendix A for specific sample type collection instructions. **Deviations from these instructions are at your expense.** Cap the sample tube once done.



5 RECORD SAMPLE DETAILS

Record the date, time (in 24-hour format), and sample type in the corresponding areas on the tube label.



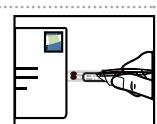
6 ASSIGN YOUR SAMPLE

Use "Assign Sample" on the left-hand navigation menu on microbio.me to associate your sample to your participant profile. **We cannot process a sample until it has been assigned.**



7 MAIL SAMPLE

Wrap the tube in absorbent tissue, such as facial tissue or paper towels, and mail it back within 48 hours of sample collection.



If you have multiple samples, it is okay to send them one at a time. If you cannot mail them all at the same time, please store the tubes at room temperature in a dry, cool, location (such as a cabinet or closet).

Please do not freeze or refrigerate samples prior to shipment, unless we have directed you otherwise.

These instructions are extremely important. Deviations will cause issues with sample processing, sequencing, and data analysis. We cannot guarantee that we will be able to process your sample and **we cannot offer replacements** if the instructions are not followed. Please do not hesitate to email us with questions at info@american gut.org.

Detailed Instructions

A desktop computer or laptop is required for Steps 1-3 and 6.

1. Registration: Register your kit at <http://microbio.me/AmericanGut/> using the kit ID and password found on the Credentials Sheet.

2. Verify Your Kit: Once registered, a 5-digit verification code will be emailed to you. When you login, confirm that the list of barcode numbers match the label numbers on the sample tubes (more than one will show up if you have ordered a kit for two or more). Check the boxes next to the barcode numbers and enter the verification code. If you did not receive a verification code or the barcode numbers listed, contact info@american gut.org immediately.

3. Create A Participant Profile: Sample options – human, animal, or environmental.

- ✓ Human: Must complete a consent form and the participant survey*. **Providing formal consent is mandatory so we can process your sample.**
- ✓ Animal: You will be asked to fill out a short survey. No consent is necessary.
- ✓ Environmental: No consent and no surveys are required. Skip Step 3 and proceed to step 4.

Hover your mouse over the [appropriate sample category](#) on the left navigation menu and click the “Add ____ source” option. (See Figure 1.)

NOTE: If there are multiple people using the sample tubes, you can repeat this step to add an additional participant.

After adding a source, next is the consent (**parental consent is required for participants under the age of 18**) and survey. After submitting your survey, you will be emailed a copy of the signed consent form (for your records).

***All human sample survey questions are optional.** If you choose not to take the survey, you may leave fields blank and proceed by clicking on “Submit”. However, your survey data is beneficial to this research and we would greatly appreciate your input.

ADDITIONAL SURVEYS:

After completing the American Gut survey, the confirmation page has links to additional surveys. These optional surveys ask for additional specific information like your diet. You may complete these surveys at a later time by clicking on your name after hovering over “Human Samples” and clicking on the survey name.

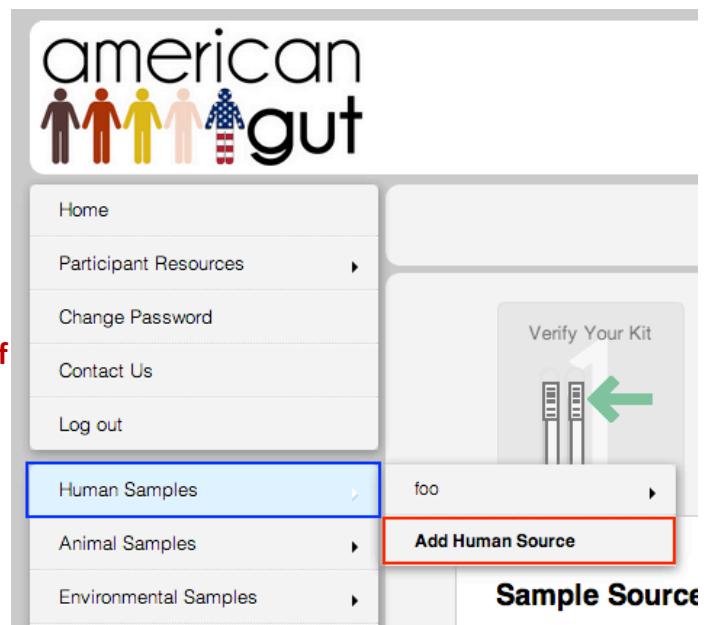


Figure 1.

Do not collect a sample until you have completed Step 3.

4. Collect Sample: See Appendix A for specific collection instructions. *It is critical to handle the tube by the red cap only* and to touch the cotton tips only to the surface of the sample being collected. If your sample is accidentally contaminated, please contact us and we will work with you on a solution. It may not be possible for us to replace samples free of charge.

5. Record Sample Details: Write the date, the time (24-hour format), and the sample type in the appropriate fields on the tube label. (see Figure 2.) If you have been provided a SWUBE/glass tube for sampling (see Figure 3.), then no information is required to be written down on the tube. For both types of tubes, **please write down and save your sample details for Step 6.**



Figure 2. Tube Recording Example:
The tube contains a specimen sample collected on June 5, at 11:00.



Figure 3. SWUBE Example.

6. Assign YOUR sample to YOUR participant profile: Click “Assign Sample” on the left navigation menu when logged into microbio.me. Select the name you want to assign a sample to, then fill out the requested form with your sample details. **Assigning your sample MUST be completed before your sample can be processed.**

7. Mail Sample: Wrap the ENTIRE sample tube (swabs inside!) in absorbent tissue (e.g. facial tissue or paper towels) and mail it back within 48 hours of sample collection. The absorbent tissue will help to keep the relative humidity within the package low. The sooner we receive your sample, the sooner we can store and prepare it for processing! If you have been provided a SWUBE, place it in the bubble wrap bag as well for additional protection. *If you cannot send your sample immediately, please do not freeze or refrigerate the sample prior to shipment unless directed otherwise.*

We recommend taking the sample to the post office for proper postage to avoid a transit delay. If samples are delayed, they will likely not produce the highest quality results. We also recommend using an additional reinforced envelope to reduce losing the sample due to damaged packaging.

All American Gut samples should be mailed to:

ATTN: Greg Humphrey, Knight Lab
BRF II Room 1220D
9500 Gilman Drive
La Jolla, CA 92093-0763

Appendix A: Sample Type Collection Instructions

1. FECAL: Rub both cotton tips on a fecal specimen (ideally, a used piece of toilet paper). Collect a small amount of biomass, saturating half the swab at maximum. More is not better! The ideal amount of biomass collected is shown in Figure 4.



Figure 4. A fecal sample showing an ideal amount of material collected. **Excessive amounts of material will result in the nonrefundable exclusion of your sample.** See also Figure 5.

It is important that the amount of material collected is small for two reasons:

1. The amount of material could bias the sample's results (e.g. mold growth might contaminate the sample).
2. Excess material cannot be handled by our processing pipeline.

Our molecular methods are incredibly sensitive and require nanograms (billionths of a gram) of DNA. Previous studies have shown that even a tiny amount of material, like in the picture above, is sufficient to assess the microbial life in your gastrointestinal tract.

The images below represent good and bad fecal samples.



Figure 5: Represents Good Sample! = Bad Sample

DIFFICULTY OBTAINING FECAL SPECIMENS: We recognize that some participants have chronic constipation. In these instances, we recommend the “catch and release” method, using no more than 2-4 paper towels, enough material to prevent fecal contact on you and to avoid clogging the toilet. If the specimen is not soft, please just rub both sides of the cotton swabs on it to get a light smear. Be certain to wash your hands afterwards!

2. ORAL: Firmly rub both sides of both cotton tips on the surface of the tongue for 20 seconds. Take great caution not to touch the inside of your cheeks, teeth, or lips. An example of the area of sampling for the tongue is shown in Figure 6.

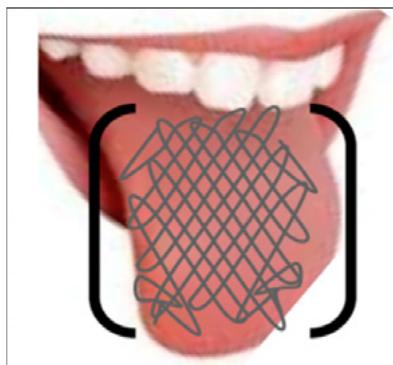


Figure 6. Example of where to rub the cotton swabs on the tongue if you are collecting an oral sample.

3. SKIN: **Do not wash the skin surface for at least two hours prior to sampling.** Firmly rub both sides of both cotton tips over the skin surface being sampled for 20 seconds. Some examples of the area of sampling are shown in Figure 7 and 8. Note that for skin samples, it is necessary to press relatively hard with the sampling tips.

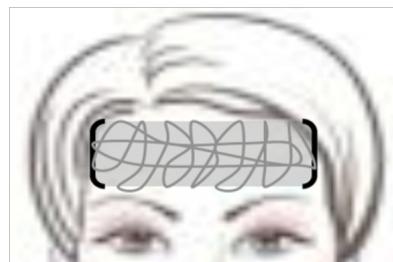


Figure 7. Example of where to rub the cotton swabs if you are collecting a forehead sample.

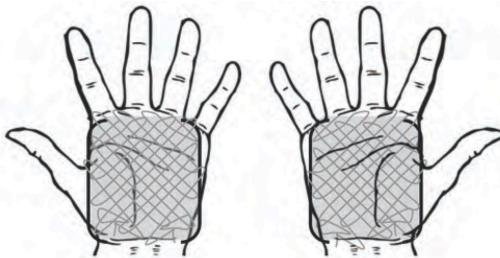


Figure 8. Example of where to rub the cotton swabs if you are collecting a sample from the palm.

NOTE: Submitting oral and skin samples will take a longer time than the expected 3 months to process and return results as we prioritize processing fecal samples.

Once you have completed sampling, cap the swabs back into the tube.

If you have further questions or issues:

First consult the FAQ section: <http://www.microbio.me/AmericanGut/faq/>.

If you cannot find the answer to your question, email info@american gut.org.