

A Complete Microbiomics Solution

Unbiased from the Beginning



About the Cover

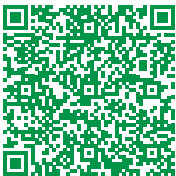


The thousands of microbiome studies performed over the past two decades have revealed the monumental impact that complex microbial communities have on human health, agriculture, and many other aspects of our lives. Among the many microbial-human interactions, none has more emotional significance than the development of an infant's microbiome through the interaction with its mother. Studies have found that the intimate bond between a mother and her baby is much deeper than we realized, with birth method, breastfeeding, and touch directly shaping the infant's microbiome and health¹⁻³. With studies investigating such profound questions about human health, all microbiome profiling experiments require strict methodology to ensure bias does not alter perceived microbial presence and abundance.

The scientists at Zymo Research are passionate about helping researchers generate reproducible, bias-free data and so have developed Microbiomics-Grade tools for each step of the microbial profiling workflow. These include well-defined mock microbial community standards, cold-free sample collection and preservation, bias-free nucleic acid extraction kits, and simplified NGS library preparation. With bias now a major concern for microbiomics researchers, many are combining the solutions of the ZymoBIOMICS® workflow to achieve the most accurate microbial profiling data for a variety of sample types.

Zymo Research is dedicated to the progression of the microbiomics field. Also, to make bias-free microbial profiling more accessible to researchers, Zymo Research utilizes its suite of Microbiomics-Grade products and techniques in the ZymoBIOMICS® 16S and Shotgun Sequencing Services to process, sequence, and analyze any microbiome samples.

ZymoBIOMICS®: Bias-Free Microbiomics Solutions



For inquiries about our ZymoBIOMICS® products and services, visit www.zymoresearch.com/pages/zymbiomics-portfolio

1. Moossavi S et al. Composition and Variation of the Human Milk Microbiota Are Influenced by Maternal and Early-Life Factors. *Cell Host & Microbe* 2019 25(2): 324-335.
2. Koenig JE, Spor A, Scalfone N, Fricker AD, Strombaugh J, Knight R, Angenent LT, Ley RE. Succession of microbial consortia in the developing infant gut microbiome. *PNAS* 2011 108(supplement 1): 4578-4585.
3. Deal DA, Chapman J, Adeyua A, Kim JH, Chen F, Whitehead TR, Lapidus A, Rokhsar DS, Lebrilla CB, German JB, Price NP, Richardson PM, Mills DA. The genome of *Bifidobacterium longum* subsp. *Infantis* reveals adaptations for milk utilization with the infant microbiome. *PNAS* 2008 105(48): 18964-18969.

A Complete Microbiomics Solution

Sample Collection & Storage

DNA/RNA Shield™ Bulk Reagent & Collection Devices

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Microbiome Standards

ZymoBIOMICS® Microbial Community Standards
ZymoBIOMICS® HMW DNA Standard
ZymoBIOMICS® Microbial Community Standards II
ZymoBIOMICS® Spike-in Control I (High Microbial Load)
ZymoBIOMICS® Spike-in Control II (Low Microbial Load)

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Nucleic Acid Extraction

ZymoBIOMICS® DNA Extraction Kits
Quick-DNA™ HMW MagBead Kit
ZymoBIOMICS® RNA Miniprep Kit
ZymoBIOMICS® DNA/RNA Miniprep Kit
HostZERO™ Microbial DNA Kit

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Library Prep & Analysis

Quick-16S™ NGS Library Prep Kit
Femto™ Bacterial DNA Quantification Kit
Select-a-Size DNA Clean & Concentrator MagBead Kit
ZymoBIOMICS® Services

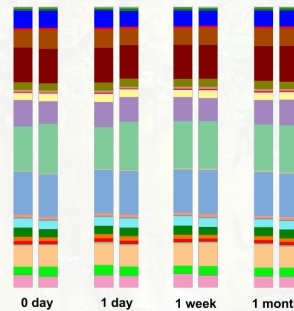
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Cold-Free Sample Collection & Storage

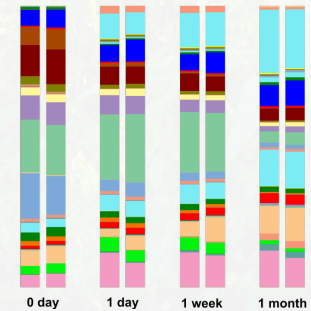
Collect Samples Anywhere, Worry-Free

The indigenous tribes of the Amazon rainforest live far from -80°C freezers and dry ice. In order to accurately assess tribes' microbiomes, researchers used DNA/RNA Shield™ to collect and preserve samples taken from stool and the fermented drink, "chicha". These unique microbial profiles were stabilized at ambient temperatures until shipped and processed at a later date.

Protected by DNA/RNA Shield™ - Accurate Composition



Not Protected Composition Changes

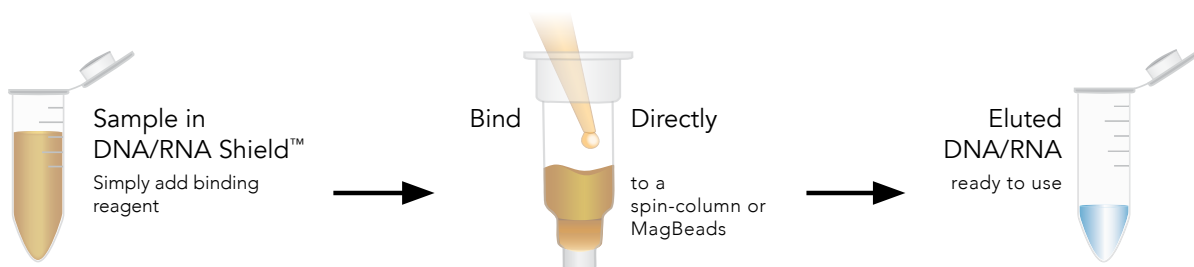


Microbial composition of stool is unchanged after one month at ambient temperature with DNA/RNA Shield™. The extracted DNA was subjected to microbial composition profiling by 16S rRNA gene targeted sequencing.

DNA/RNA Shield™

- **Microbiome DNA/RNA Preservative:** Preserves genetic integrity and expression profiles of samples at ambient temperatures.
- **Safe Transport:** Inactivates infectious agents (virus, bacteria, parasites, yeasts).
- **Streamlined Workflow:** DNA and RNA can be isolated directly from preserved sample without lengthy precipitation or reagent removal steps.

Streamlined Workflow



Plug samples directly into purification - no reagent removal

Safe Sample Handling

DNA/RNA Shield™: Complete Pathogen Inactivation



BY

Eurovir® Hygiene-Labor
Antivirale Validierung & Rabies

DNA/ RNA Shield™ effectively inactivates pathogens. This includes tough-to-lyse microbes or viruses without the need for additional steps, such as heat-treatment, homogenization, or alcohol sterilization. DNA/RNA Shield™ has been rigorously tested to ensure inactivation of pathogens including:

Bacteria

B. subtilis, *E. faecalis*, *E. coli*, *L. fermentum*, *L. monocytogenes*, *M. tuberculosis*, *P. aeruginosa*, *S. enterica*, *S. aureus*, *S. pneumoniae*, *X. fastidiosa*

Viruses

Parvovirus, Chikungunya virus, Dengue virus, Ebola virus, Herpes simplex virus-1, Herpes simplex virus-2, Influenza A, Rhinovirus, MERS-coronavirus, West Nile virus

Yeast & Eukaryotes

C. albicans, *C. neoformans*, *S. cerevisiae*, *P. malariae*



Transport



Handling



Processing

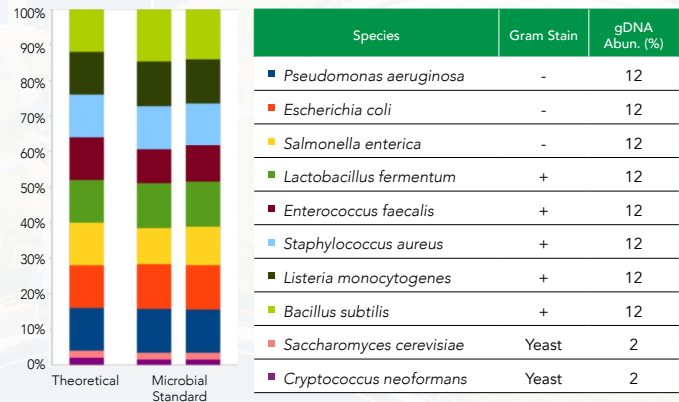
Product	Cat. No.	Size
DNA/RNA Shield™	R1100-50 R1100-250	50 ml 250 ml
DNA/RNA Shield™ - Lysis Tube (Microbe)	R1103	50 pack
DNA/RNA Shield™ - Lysis Tube (Microbe) with Swab	R1104	50 tubes/50 swabs
DNA/RNA Shield™ Collection Tube w/Swab	R1106	10 pack (1 ml fill)
	R1107	50 pack (1 ml fill)
	R1108	10 pack (2 ml fill)
	R1109	50 pack (2 ml fill)
DNA/RNA Shield™ - Fecal Collection Tube	R1101	10 pack
DNA/RNA Shield™ Saliva Collection Kit	R1210	1 x 2 ml fill

Mock Microbial Community Standards

Precise Composition for Reproducible Data

The ZymoBIOMICS® Microbial Community Standard is comprised of eight bacterial and two fungal species, representing a wide range of cell wall toughness.

Researchers used the ZymoBIOMICS® Microbial Community Standard to ensure accurate and unbiased microbial profiling of various points during the sanitization process at the world's largest water reclamation facility¹.



The ZymoBIOMICS® Microbial Community Standard contains three easy-to-lyse bacteria, five tough-to-lyse bacteria, and two tough-to-lyse yeasts.

A Comprehensive Mock Community Portfolio

ZymoBIOMICS® Standards	Microbial Community Standard		Microbial Community Standard II		Spike-in Control		HMW DNA Standard
Formats	Microbial Cell	Isolated DNA	Microbial Cell	Isolated DNA	High Microbial Load	Low Microbial Load	Isolated DNA
Detect Bias In:	Complete Workflow & DNA Extraction	Library Prep & Sequencing	Complete Workflow & DNA Extraction	Library Prep & Sequencing	Complete Workflow & DNA Extraction	Complete Workflow & DNA Extraction	Long-read Library Prep & Sequencing
Recommended Use	General benchmarking and microbiome profiling control		Assess detection limit and sensitivity		Absolute quantification of high bacterial-load samples (e. g. feces)	Absolute quantification of low bacterial-load samples (e. g. sputum)	General benchmarking and microbiome profiling control for long-read sequencing

Accurately Define Composition

	ZymoBIOMICS® Microbial Standards	Supplier A
Abundance Variation	< 15%	< 1,000%
Foreign Contaminants	< 0.01%	< 10%
Include Both Bacteria and Fungi	✓	✗
Strict Manufacturing QC	✓	✗

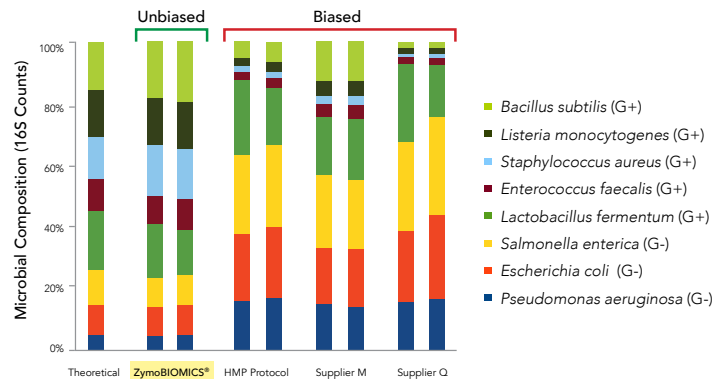
Ensure Accurate Microbial Profiling

ZymoBIOMICS® Microbial Community Standard

- **Accurate composition:** composition cross-validated using multiple types of measurements.
- **Assessing bias in DNA isolation:** containing microbes of varying size and cell wall recalcitrance (8 bacteria and 2 yeasts).
- **Microbiomics QC:** Ideal for a complete microbiome workflow quality control.

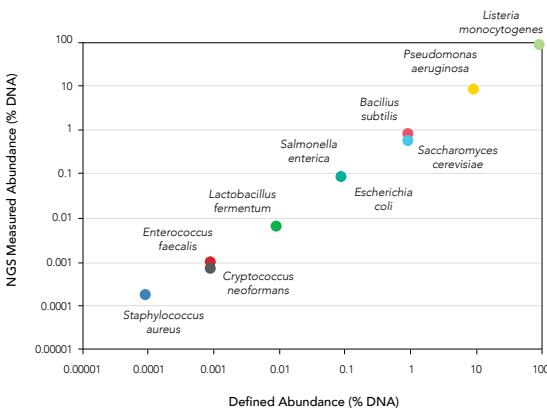
Identify and Eliminate Bias

The ZymoBIOMICS® Microbial Community Standard was used to compare different DNA extraction protocols. DNA samples were profiled by 16S rRNA gene targeted sequencing.



ZymoBIOMICS® Microbial Community Standard II

- **Log abundance distribution:** assess detection limit over a broad range (10^2 to 10^8 cells).
- **Accurate Composition:** cross-validated with multiple measurements.
- **Microbiomics QC:** Quality control for microbiome profiling and pathogen identification.



Validated Log-Abundance Distribution

The ZymoBIOMICS® Microbial Community Standards II has an accurate log-abundance distribution as validated with shotgun sequencing analysis.

Product	Cat. No.	Size
ZymoBIOMICS® Microbial Community Standard	D6300	10 preps
ZymoBIOMICS® Microbial Community DNA Standards	D6305 D6306	200 ng 2,000 ng
ZymoBIOMICS® Microbial Community Standard II (Log Distribution)	D6310	10 preps
ZymoBIOMICS® Microbial Community DNA Standard II (Log Distribution)	D6311	220 ng
ZymoBIOMICS® HMW DNA Standards	D6322	5,000 ng

Quantify Absolute Cell Numbers

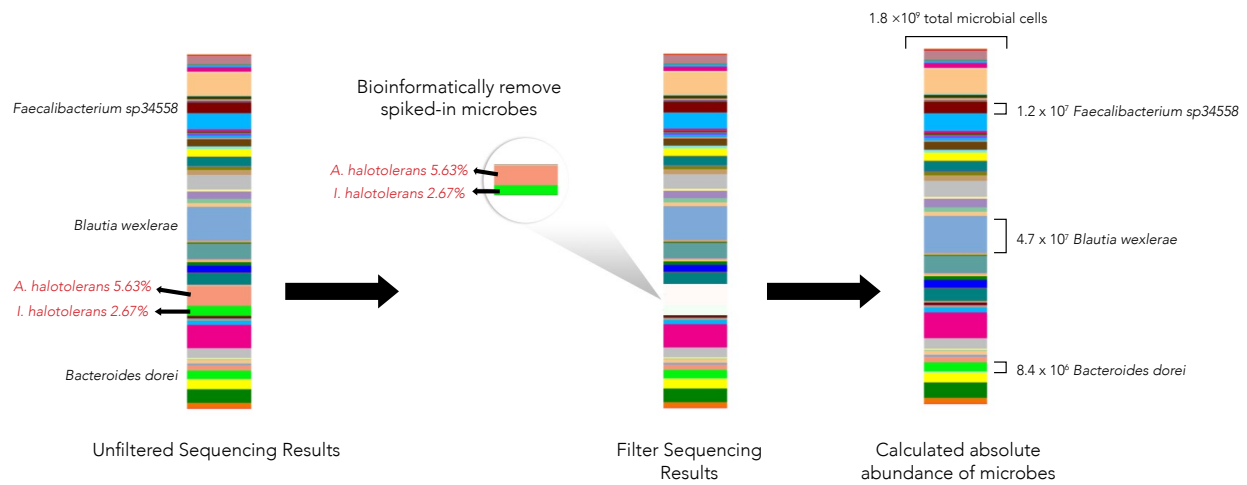
ZymoBIOMICS® Spike-in Control I (High Microbial Load)

- **Absolute Quantification:** Enables cell number measurements using Next-Gen Sequencing.
- **In situ Quality Control:** Ensures each sample is quantified accurately.
- **Unique composition:** Comprised of two microbes alien to the human microbiome.

Defined Composition

Spike-in Species	Per Prep (20 µl)		
	Cells	16S Copies	Total DNA (ng)
<i>Imtechella halotolerans</i>	2×10^7	6.0×10^7	67.2
<i>Allobacillus halotolerans</i>	2×10^7	1.4×10^8	58.2

Quantify Absolute Abundance of Microbes



The ZymoBIOMICS® Spike-in Control I was added to a fecal sample. Total DNA was then extracted using the ZymoBIOMICS® DNA Miniprep Kit, a library was prepared and then analyzed by 16S targeted Next-Gen Sequencing. From the resulting data, the percentage of *Imtechella halotolerans* and *Allobacillus halotolerans* in the total sample composition was then calculated. The resulting percentage was paired to the defined Spike-in I cell input and used to calculate the absolute abundance for organisms in the sample based their sample composition percentages.

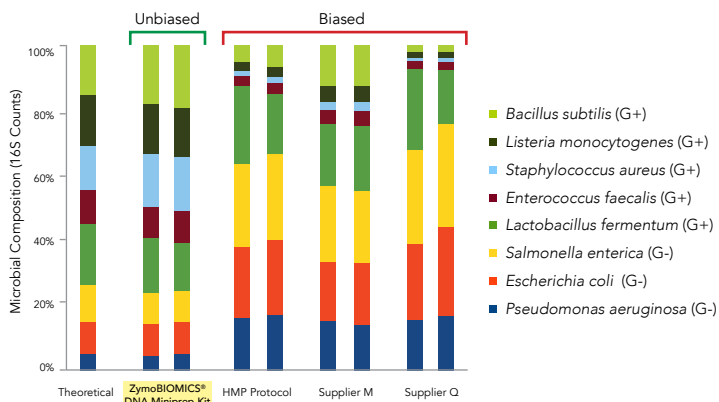
Product	Cat. No.	Size
ZymoBIOMICS® Spike-in Control I (High Microbial Load)	D6320 D6320-10	25 preps 250 preps
ZymoBIOMICS® Spike-in Control I (Low Microbial Load)	D6321 D6321-10	25 preps 250 preps

Unbiased Extraction from Any Sample

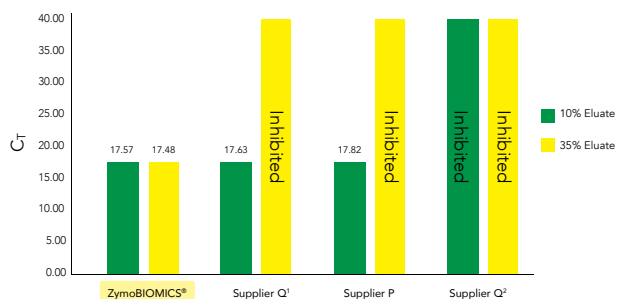
ZymoBIOMICS® DNA Extraction Kits (feces, soil, swabs, water, biofilms, body fluids, etc.)

- **Validated Unbiased for Microbiome Measurements:** Unbiased cellular lysis validated using the ZymoBIOMICS® Microbial Community Standard.
- **Inhibitor-Free DNA from Any Sample:** Isolate ultra-pure DNA ready for any downstream application.
- **Certified Low Bioburden:** Boost your detection limit for low abundance microbes.

Unbiased Lysis



Ultra-pure DNA from Inhibitor-Rich Samples



DNA was extracted from ZymoBIOMICS® Microbial Community Standard using four different DNA extraction methods (ZymoBIOMICS® DNA Miniprep Kit, Human Microbiome Project Protocol, Supplier M, and Supplier Q) and analyzed using 16S rRNA gene sequencing. The ZymoBIOMICS® DNA Miniprep Kit provides accurate representation of the organisms extracted.

The ZymoBIOMICS® DNA Miniprep Kit provides inhibitor-free DNA even when challenged with extremely inhibitor rich samples. Real-time PCR was used to evaluate eluates recovered using the ZymoBIOMICS® DNA Miniprep Kit, and kits from Suppliers Q¹, P, and Q². Delayed and/or no amplification indicates PCR inhibition from inefficient inhibitor removal.



CERTIFIED
Ultra-Clean

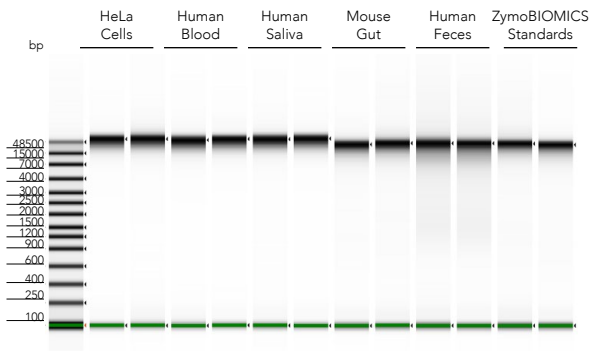
Low Bioburden | DNA Free

Product	Cat. No.	Size
ZymoBIOMICS® DNA Miniprep Kit	D4300	50 preps
ZymoBIOMICS® DNA Microprep Kit	D4301	50 preps
ZymoBIOMICS® 96 DNA Kit (includes ZR BashingBead™ Lysis Rack)	D4303	2 x 96 preps
ZymoBIOMICS® 96 DNA Kit (includes ZR BashingBead™ Lysis Tubes)	D4309	2 x 96 preps
ZymoBIOMICS® 96 MagBead DNA Kit (includes ZR BashingBead™ Lysis Tubes)	D4308	2 x 96 preps
ZymoBIOMICS® 96 MagBead DNA Kit (includes ZR BashingBead™ Lysis Rack)	D4302	2 x 96 preps

High Molecular Weight DNA & Ultra-Pure RNA

Quick-DNA™ HMW MagBead Kit

- **Third-Generation Sequencing Ready:** Optimized for long read sequencing assays.
- **High Quality HMW DNA:** Extract high molecular weight DNA > 100 kb size from any sample type.
- **Unmatched Recovery:** Highest DNA yield and purity.



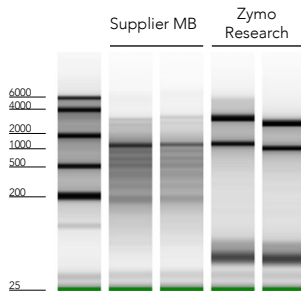
High Quality DNA from Any Sample Type

The Quick-DNA™ HMW MagBead Kit was used to extract high molecular weight DNA from a variety of samples including human feces and the ZymoBIOMICS® Microbial Community Standard. Quality was assessed using Agilent 2200 TapeStation®.

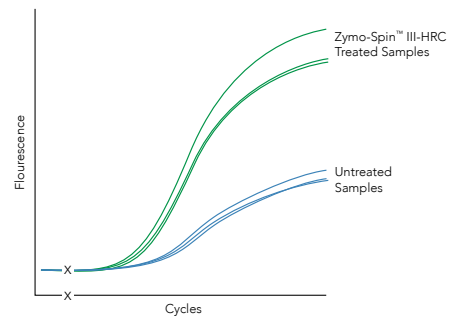
ZymoBIOMICS® RNA Miniprep Kit

- **Unbiased Cellular Lysis:** ZymoBIOMICS® innovative lysis system enables efficient and unbiased lysis of microbes including Gram positive/negative bacteria, protozoans, algae, viruses etc.
- **Ultra-Pure:** Total RNA (including small/microRNAs) is inhibitor-free and ready for use in any downstream application.
- **High Sensitivity:** Increased detection limit of very low abundance organisms.

Ultra-pure RNA from Inhibitor-rich Samples



Human stool RNA isolated with the ZymoBIOMICS® RNA Miniprep Kit is higher quality (right); compared to Supplier MB procedures (left). Quality assessed by Agilent 2200 TapeStation®.



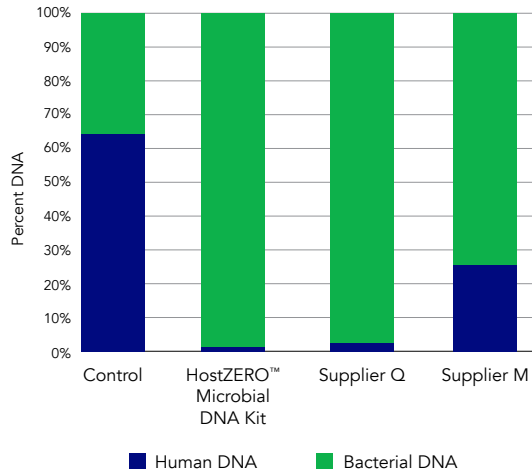
Total RNA isolated from human stool with or without inclusion of the Zymo-Spin™ III-HRC Spin Filter during the ZymoBIOMICS® RNA Miniprep Kit protocol. Earlier amplification cycles indicate complete removal of PCR inhibitors.

Product	Cat. No.	Size
Quick-DNA™ HMW MagBead Kit	D6060	96 preps
ZymoBIOMICS® RNA Miniprep Kit	R2001	50 preps
ZymoBIOMICS® DNA/RNA Miniprep Kit	R2002	50 preps

Simple Host DNA Depletion

HostZERO™ Microbial DNA Kit

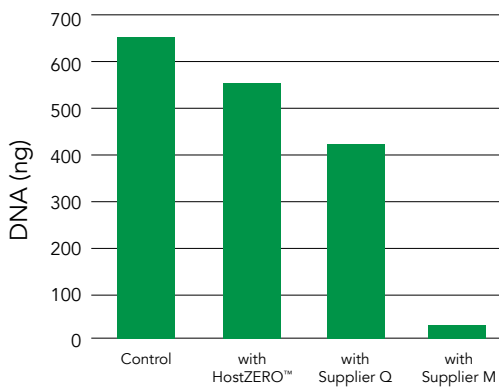
- **Depletes Host DNA:** ≥90% depletion in applicable sample types.
- **Preserves Microbial DNA:** ≥85% recovery of microbial DNA and minimum impact on microbiome profile.
- **Simple and Fast:** Only 30 minutes of hands-on time.



Best Depletion of Host DNA

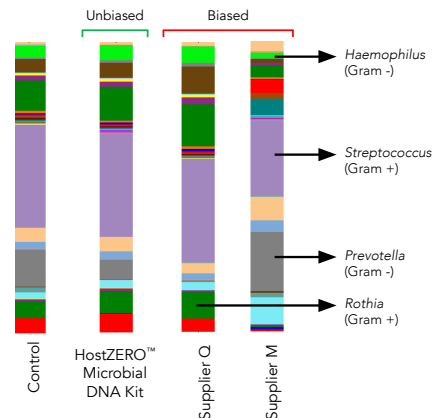
The HostZERO™ Microbial DNA Kit depletes host DNA. The same human saliva sample was processed using the HostZERO™ Microbial DNA Kit or kits from Suppliers Q and M. Triplicate samples of purified DNA were evaluated by Real-time PCR. The composition of the DNA is shown in terms of relative bacterial and human DNA abundance. The control sample was processed with the ZymoBIOMICS® DNA Microprep Kit, which extracts total DNA from the sample without host DNA depletion.

Highest Recovery of Bacterial DNA



Bacterial DNA is efficiently recovered with HostZERO™ technology. The same human saliva sample was processed using the HostZERO™ Microbial DNA Kit or kits from Suppliers Q and M. Triplicate samples of purified DNA were evaluated by Real-time PCR. The control sample was processed with the ZymoBIOMICS® DNA Microprep Kit, which extracts total DNA from the sample without host DNA depletion.

Preserves the Microbial Profile



The microbial composition is maintained in samples processed with the HostZERO™ Microbial DNA Kit. The same human saliva sample was processed using the HostZERO™ Microbial DNA Kit or kits from Suppliers Q and M. Triplicate samples of purified DNA were analyzed using 16S rRNA gene targeted sequencing using primers targeting the V3-V4 region. The resulting amplicons were sequenced on the Illumina MiSeq® (2x300bp). The control sample was processed with the ZymoBIOMICS® DNA Microprep Kit, which extracts total DNA from the sample without host DNA depletion.

Product	Cat. No.	Size
HostZERO™ Microbial DNA Kit	D4310	50 preps

Fast and Unbiased 16S Library Prep

Quick-16S™ NGS Library Prep Kit

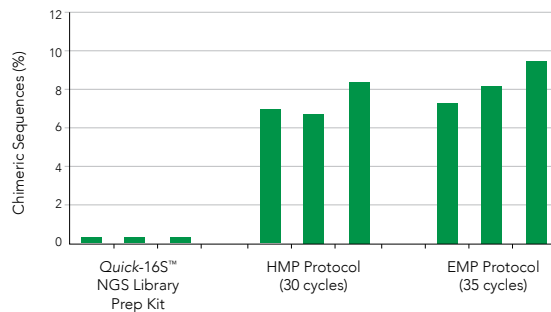
- **Fast & Simple:** Only 1.5 hours of hands-on time. No TapeStation® analyses or AMPure® clean-ups.
- **Accurate:** Real-time PCR reduces PCR chimera formation by up to 10 times.
- **Increased Coverage:** Novel primers increase phylogenetic coverage of bacteria and archaea, enabling species-level resolution for human microbiome profiling.



Fastest 16S Library Prep

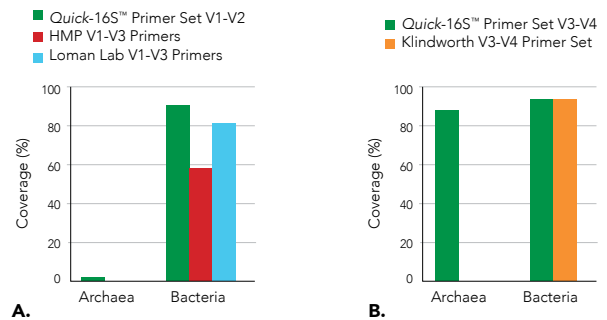
The Quick-16S™ NGS Library Prep Kit is >2.5 times faster than the conventional 16S library prep method. The Quick-16S™ Kit simplifies the 16S library prep workflow by quantifying libraries using qPCR, instead of TapeStation® analyses, and by using a single-tube library cleanup.

Minimize PCR Chimera Formation



The Quick-16S™ NGS Library Prep Kit minimizes PCR chimera formation compared to two common protocols: Human Microbiome Project (HMP) and Earth Microbiome Project (EMP). Equivalent amounts of the same fecal DNA sample were used as input. Chimeric sequences were predicted with Uchime (<https://www.drive5.com/uchime>).

Best Phylogenetic Coverage



A. The Quick-16S™ Primer Set V1-V2 includes coverage of common human-associated microbes, including *Bifidobacterium*, *Propionibacterium*, and *Chlamydia*, which are missed in common V1-V2 or V1-V3 primers.

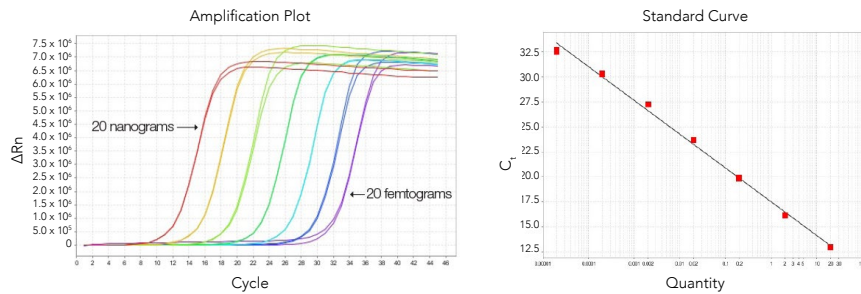
B. The Quick-16S™ Primer Set V3-V4 provides up to 87% coverage for archaea, organisms commonly found in the human gut. The common V3-V4 primers provide 0% coverage for archaea.

Product	Cat. No.	Size
Quick-16S™ NGS Library Prep Kit	D6400	96 rxns

Precise Quantification & Size Selection

Femto™ DNA Quantification Kits

- **Femtogram Sensitivity:** Quantify as little as 20 femtograms of DNA.
- **Reliable Quantification:** High specificity and sensitivity for bacterial, fungal, & human DNA.
- **Fast and simple:** Add samples to the PreMix and quantify.

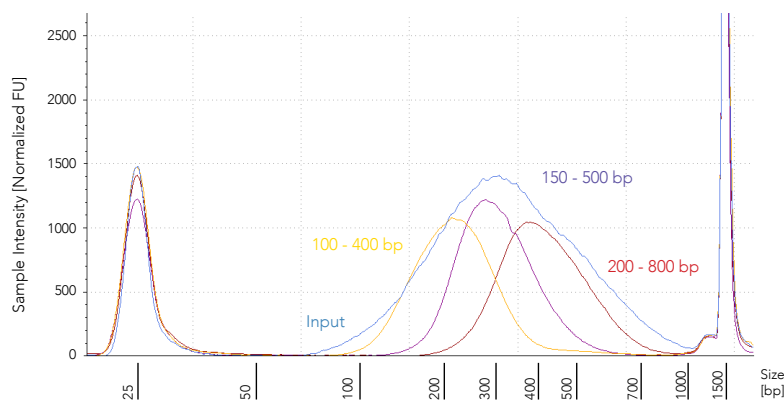


Reliable Quantification

Reliable standards for the qualification of bacterial DNA. Bacterial DNA Standards (measured in duplicates) comprise a 10-fold dilution series ranging from 20 ng to 20 fg.

Select-a-Size DNA Clean & Concentrator® MagBead Kit

- **Tunable:** Size selection can be tuned from 100 bp to 1000 bp with left, right, or double size selection.
- **Ultra-Pure:** 10 μl elutions are ready for Next Generation Sequencing, etc.
- **Automation Ready:** Scripts and automation support readily available.



Easy Size Selection

Select-a-Size DNA Clean & Concentrator® MagBead Kit allows for adjustable size selection. Exemplary size selections (using 2 μg of sonicated DNA) were analyzed using the Agilent 2200 TapeStation® system.

Product	Cat. No.	Size
Femto™ Human DNA Quantification Kit	E2005	100 rxns
Femto™ Bacterial DNA Quantification Kit	E2006	100 rxns
Femto™ Fungal DNA Quantification Kit	E2007	100 rxns
Select-a-Size DNA Clean & Concentrator® MagBead Kt	D4084 D4085	10 ml 50 ml

Comprehensive Sequencing Services

ZymoBIOMICS® Services

A Complete Solution for Unbiased Microbiome Profiling

- Simply submit samples and receive a comprehensive, customizable, and user-friendly report.
- Unbiased & contamination-free sample processing validated using microbial standards.

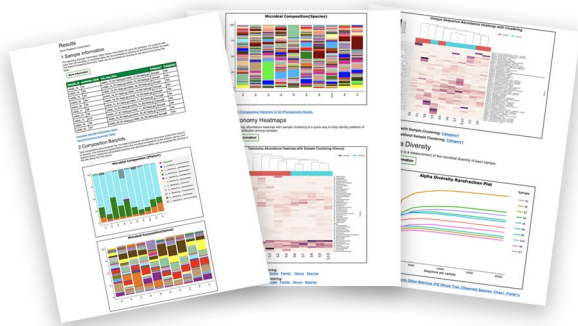
Quick Turnaround

- Results in ≤ 2 weeks for 16S rRNA gene sequencing.
- Results in ≤ 6 weeks for shotgun metagenomic sequencing.

Superior Taxonomic Resolution

- Species-level resolution with 16S rRNA gene sequencing.
- Strain-level resolution with shotgun metagenomic sequencing.

End-to-End Microbiomics Services, Including Bioinformatics Analysis.



Comprehensive and User-Friendly Report Includes:

- Composition Bar Plots
- Alpha-Diversity
- Statistical Analysis
- Taxonomy Heatmap
- Beta-Diversity
- Biomarker Discovery (LEfSe)



Visit www.zymoresearch.com/pages/zymbiomic-services for a custom microbiomics services quote.

Microbiomics Products

Product	Cat. No.	Size
DNA/RNA Shield™	R1100-50 R1100-250	50 ml 250 ml
DNA/RNA Shield™ - Lysis Tube (Microbe)	R1103	50 pack
DNA/RNA Shield™ - Lysis Tube (Microbe) with Swab	R1104	50 tubes/50 swabs
DNA/RNA Shield™ Collection Tube w/Swab	R1106 R1107 R1108 R1109	10 pack (1 ml fill) 50 pack (1 ml fill) 10 pack (2 ml fill) 50 pack (2 ml fill)
DNA/RNA Shield™ - Fecal Collection Tube	R1101	10 pack
DNA/RNA Shield™ Saliva Collection Kit	R1210	1 x 2 ml fill
ZymoBIOMICS® Microbial Community Standard	D6300	10 preps
ZymoBIOMICS® Microbial Community DNA Standards	D6305 D6306	200 ng 2,000 ng
ZymoBIOMICS® Microbial Community Standard II (Log Distribution)	D6310	10 preps
ZymoBIOMICS® Microbial Community DNA Standard II (Log Distribution)	D6311	220 ng
ZymoBIOMICS® HMW DNA Standards	D6322	5,000 ng
ZymoBIOMICS® Spike-in Control I (High Microbial Load)	D6320 D6320-10	25 preps 250 preps
ZymoBIOMICS® Spike-in Control II (Low Microbial Load)	D6321 D6321-10	25 preps 250 preps
ZymoBIOMICS® DNA Miniprep Kit	D4300	50 preps
ZymoBIOMICS® DNA Microprep Kit	D4301	50 preps
ZymoBIOMICS® 96 DNA Kit (includes ZR BashingBead™ Lysis Rack)	D4303	2 x 96 preps
ZymoBIOMICS® 96 DNA Kit (includes ZR BashingBead™ Lysis Tubes)	D4309	2 x 96 preps
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Quick-16S™ NGS Library Prep Kit	D6400	96 rxns
Femto™ Human DNA Quantification Kit	E2005	100 rxns
Femto™ Bacterial DNA Quantification Kit	E2006	100 rxns
Femto™ Fungal DNA Quantification Kit	E2007	100 rxns
Select-a-Size DNA Clean & Concentrator® MagBead Kt	D4084 D4085	10 ml 50 ml



ZYMO RESEARCH

The Beauty of Science is to Make Things Simple®

Cat. No. B1007 Version 04

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