INSTRUCTION MANUAL

ZymoBIOMICS® Gut Microbiome Standard

Catalog No. D6331

Highlights

- **True to Life:** comprised of 21 different strains to mimic the human gut microbiome
- **Accurate Composition:** allows for benchmarking and validation of NGS microbiome workflows
- **Cross Kingdom Representation:** includes Bacteria, Fungi, and Archaea

Contents

ZYMO RESEARCH CORP.

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Satisfaction of all Zymo Research products is guaranteed. If you are dissatisfied with this product, please call 1-888-882-9682.

Notes:

[1] For short-term storage or regular use, -20°C may be used.

[2] The microbial composition of each lot was measured by shotgun metagenomic sequencing. The results (including the composition, impurities and abundance deviation) can be accessed through the Certificate of Analysis based on the lot number on the website.

[3] The reference composition will depend on the sequencing method. For 16S targeted sequencing, use the 16S copy percentage as a reference. For shotgun sequencing data based on read depth/coverage, use the genome copy percentage as a reference.

This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

Product Contents:

Product Name	D6331 (10 Preps)	Storage Temperature[1]
ZymoBIOMICS® Gut Microbiome Standard	750 µl	-80°C

Specifications:

Biosafety: this product contains no biohazard as microbes have been fully inactivated.

Reference genomes and 16S&18S rRNA genes:
https://s3.amazonaws.com/zymo-files/BioPool/D6331.refseq.zip

Storage solution: 2X DNA/RNA Shield™ (Cat. No. R1200-125).

Total cell concentration: ~9.0 x 10^9 cells/ml

Impurity level: < 0.01% foreign microbial DNA

Relative-abundance deviation in average: <15%.

Microbial composition[2]: Table 1 shows the theoretical microbial composition of the standard.

Table 1: Microbial Composition

Species	Theoretical Composition[3] (%)				
	Genomic DNA	16S Only	16S & 18S	Genome Copy	Cell Number
Faecalibacterium prausnitzii	14	17.63	15.96	14.77	14.82
Veillonella rogosae	14	15.87	14.37	19.94	20.01
Roseburia hominis	14	9.89	8.95	12.43	12.47
Bacteroides fragilis	14	9.94	9.00	8.33	8.36
Prevotella corporis	6	4.98	4.51	6.26	6.28
Bifidobacterium adolescentis	6	8.78	7.95	8.83	8.86
Fusobacterium nucleatum	6	7.49	6.79	7.53	7.56
Lactobacillus fermentum	6	9.63	8.72	9.68	9.71
Clostridioides difficile	1.5	2.62	2.37	1.10	1.10
Akkermansia muciniphila	1.5	0.97	0.87	1.62	1.62
Methanobrevibacter smithii	0.1	0.066	0.060	0.17	0.17
Salmonella enterica	0.01	0.009	0.008	0.007	0.0065
Enterococcus faecalis	0.001	0.0009	0.0008	0.0011	0.0011
Clostridium perfringens	0.0001	0.0002	0.0002	0.00009	0.00009
Escherichia coli (JM109)	2.8	2.53	2.29	1.82	1.83
Escherichia coli (B-3008)	2.8	2.53	2.29	1.82	1.82
Escherichia coli (B-2207)	2.8	2.29	2.07	1.64	1.65
Escherichia coli (B-766)	2.8	2.31	2.09	1.66	1.66
Escherichia coli (B-1109)	2.8	2.46	2.23	1.77	1.77
Candida albicans	1.5	N/A	3.11	0.31	0.16
Saccharomyces cerevisiae	1.4	N/A	6.35	0.32	0.16

Product Description:

Microbial composition profiling techniques powered by Next-Generation Sequencing are becoming routine in microbiomics and metagenomics studies. It is well known that these analytical techniques can suffer from bias and errors in every step of the workflow, including DNA extraction, library preparation, sequencing and bioinformatics analysis. To assess the performance of different microbiomics workflows, there is an urgent need in the field for reliable reference materials, e.g. a mock microbial community with a defined composition.

ZymoBIOMICS® Gut Microbiome Standard is a mixture of 18 bacterial strains, 2 fungal strains, and 1 archaeal strain in staggered abundances to mimic a true gut microbiome. The standard presents multiple challenges for NGS pipelines, such as tough-to-lyse Gram-positive bacteria (e.g. Roseburia hominis) to test lysis efficiency, genomes with a wide range of GC content to test sequencing coverage bias, low-abundance pathogenic organisms for detection limit assessment and 5 different strains of *E. coli* to test taxonomic resolution. These challenge points can be used to expose artifacts, errors, and bias in microbiomics or metagenomics workflows. Serving as a defined input, this standard can be used to guide construction and optimization of entire workflows or as a quality control tool for inter-lab studies.

The microbial standard is accurately characterized and contains negligible impurity (< 0.01%). It was constructed by pooling cells from pure cultures of 21 microbial strains. The cells from each pure culture were quantified before pooling. After mixing, the microbial composition was confirmed using NGS-based sequencing (Figure 1).

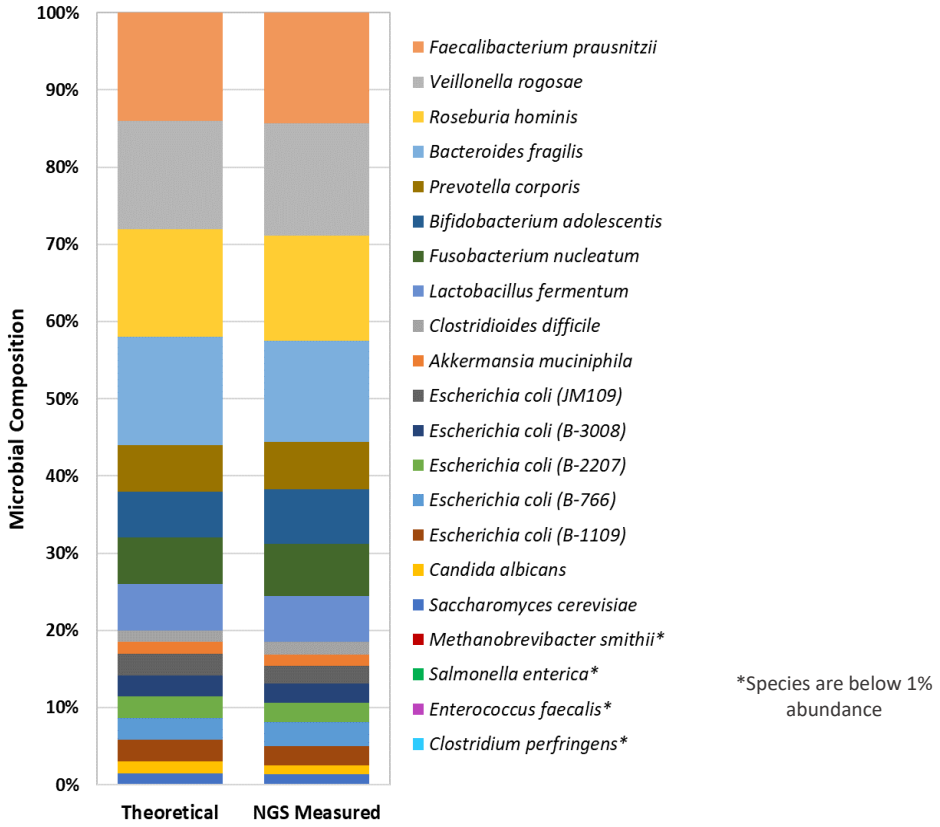

Figure 1. The microbial composition of the standard measured by NGS shotgun sequencing as compared to the defined composition. The microbial composition of the standard was confirmed using Illumina® shotgun sequencing. Genomic DNA was extracted using the ZymoBIOMIC® DNA Miniprep. Library preparation was performed using an in-house protocol. Shotgun sequencing was performed using Illumina HiSeq™ or MiSeq™. Microbial abundance was estimated based on the number of reads that were mapped to references genomes of the organisms.

For **Technical Assistance**, please contact **Zymo** at 1-888-882-9682 or E-mail tech@zymoresearch.com.

Notes:

Strain Information:

Table 2: Strain information

Species	Strain ID	Genome Size (Mb)	16/18S Copy Number	GC Content (%)	Gram Stain
Faecalibacterium prausnitzii	AP34BHI	2.914	6	57.8	+
Veillonella rogosae	AC2811 AN NA 2	2.158	4	39.0	-
Roseburia hominis	OB EAV1 11 DCM	3.463	4	48.7	+/-
Bacteroides fragilis	OB EAV1 11 D6 FAA	5.167	6	43.3	-
Prevotella corporis	OB21 FMU 4	2.947	4	44.4	-
Bifidobacterium adolescentis	LMG 10502	2.090	5	59.2	-
Fusobacterium nucleatum	2/1/50A	2.448	5	27.0	-
Lactobacillus fermentum	B-1840	1.905	5	52.3	+
Clostridioides difficile	P4D3A1-1	4.209	12	28.8	+
Akkermansia muciniphila	OB21 FAA NB 28	2.851	3	55.5	-
Methanobrevibacter smithii	DSM 861	1.853	2	31.0	+
Salmonella enterica	B-4212	4.760	7	52.2	-
Enterococcus faecalis	IP101412 AER FAA 2	2.845	4	37.5	+
Clostridium perfringens	OB21 TSA 19	3.436	10	28.3	+
Escherichia coli	JM109	4.729	7	50.9	-
Escherichia coli	B-3008	4.739	7	50.9	-
Escherichia coli	B-2207	5.234	7	50.7	-
Escherichia coli	B-766	5.191	7	50.8	-
Escherichia coli	B-1109	4.875	7	50.5	-
Candida albicans	IHEM 3108	14.68	55[1]	33.6	n/a
Saccharomyces cerevisiae	Y-567	13.30	109[1]	38.3	n/a

[1] 18S rRNA gene copy numbers in a haploid genome of the two strains of *Saccharomyces cerevisiae* and *Candida albicans* were estimated based on read depth information from mapping shotgun sequencing data

Protocol:

1. Thaw the microbial standard completely. Mix thoroughly by vortex to ensure cells are evenly resuspended.

2. Use 75 µl per prep for DNA extraction[1]. The standard is recommended to be processed alongside experimental samples throughout the entire microbiomics workflow as a positive control.

   For validated bead beating protocols with the ZymoBIOMICS DNA Miniprep kit, see Appendix A.

Bioinformatics Analysis Recommendations

1. Assessing accuracy of taxonomy identification

The value of sequencing-based microbiome studies lies in their ability to identify microbial organisms without the need for culturing. Therefore, the accuracy of taxonomic identification is critical. The user can use the ZymoBIOMICS® Gut Microbiome Standard to compare workflow results with the theoretical composition (Table 1) to assess taxonomic identification accuracy. This will allow for the assessment of taxonomic resolution limits, and false positive and false negative rates of the workflow. False positives may be introduced by contaminations during wet-lab processing, chimeric sequences during library preparation, sequencing errors, demultiplexing errors and defects during bioinformatics analysis. The standard is certified to contain less than 0.01% of foreign contaminants. Therefore, any alien taxa present at >0.01% in the analysis can be attributed to contaminants introduced by the processing workflow.

2. Assessing bias in composition profiling

Accurately determining microbial composition of a sample is critical for conducting microbiome studies. Both wet-lab and dry-lab processes can introduce bias into the composition results samples. To determine biases introduced during wet-lab procedures, an accurate and unbiased method of bioinformatical analysis is needed. We have found that direct read-mapping against reference genomes (or against reference 16S &18S sequences, rather than assigning sequences to taxonomies, is a straightforward and accurate way to infer microbial composition of the standard from sequencing data. The reference sequences of this standard can be found in the “Specifications” (page 1) section.

Notes:

[1] The expected yield for one prep (75 µl) of the standard is approximately 1µg. Yields significantly lower than 1 µg may suggest inefficient lysis during DNA extraction.

Appendix A: Lysis Protocols

Inefficient lysis during DNA extraction can cause bias in microbiome results. Below are protocols validated by Zymo Research to have minimal lysis bias with the ZymoBIOMICS® DNA Miniprep Kit:

MP FastPrep-24™ Bead Beating Protocol:
2 ml BashingBead Tubes
Maximum of 20 tubes. The weight of > 20 tubes may cause a system error.

1. 1 minute on at max speed
2. 5 minutes rest
3. Repeat cycle 5 times for a total of 5 minutes of bead beating

Biospec Mini-Beadbeater-96 Bead Beating Protocol:
2 ml BashingBead Tubes

1. 5 minutes on at Max RPM
2. 5 minutes rest
3. Repeat cycle 4 times for a total of 20 minutes of bead beating

Biospec Mini-Beadbeater-96 Bead Beating Protocol:
96-well Lysis Rack

1. 5 minutes on Max RPM
2. 5 minutes rest
3. Repeat cycle 8 times for a total of 40 minutes of bead beating

Bertin Precellys Evolution Bead Beating Protocol:
2 ml BashingBead Tubes

1. 1 minute on at 9,000 RPM
2. 2 minutes rest
3. Repeat cycle 4 times for a total of 4 minutes of bead beating

Vortex Genie with Horizontal-(24) Microtube Adaptor Bead Beating Protocol:
2 ml BashingBead Tubes
Maximum of 18 tubes. The weight of > 18 tubes will slow the mechanism and introduce bias.

1. 40 minutes of continuous bead beating (max of 18 tubes per adaptor)

Ordering Information

Product Description	Size	Catalog No.
ZymoBIOMICS® Gut Microbiome Standard	10 preps	D6331

Related Products

Product Description	Size	Catalog No.
ZymoBIOMICS® DNA Miniprep Kit	50 preps	D4300
ZymoBIOMICS® Microbial Community Standard	10 preps	D6300
ZymoBIOMICS® Microbial Community DNA Standard (200ng)	200 ng	D6305
ZymoBIOMICS® Microbial Community DNA Standard (2000ng)	2000 ng	D6306
ZymoBIOMICS® Microbial Community Standard II (Log Distribution)	10 preps	D6310
ZymoBIOMICS® Microbial Community DNA Standard II (Log Distribution)	220 ng	D6311
ZymoBIOMICS® Spike-in Control I (High Microbial Load)	25 preps	D6320
ZymoBIOMICS® Spike-in Control I (High Microbial Load)	250 preps	D6320-10
ZymoBIOMICS® Spike-in Control II (Low Microbial Load)	25 preps	D6321
ZymoBIOMICS® Spike-in Control II (Low Microbial Load)	250 preps	D6321-10


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