

## Produktinformation



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Diagnostik & molekulare Diagnostik
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## Zuschläge

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- Trockeneiszuschlag
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- Expressversand

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# INSTRUCTION MANUAL

## ZymoBIOMICS<sup>™</sup> HMW DNA Standard Catalog No. D6322

## Highlights

- Long-Read Sequencing Ready: Ideal to benchmark 3<sup>rd</sup> generation sequencing and metagenomic measurements (*e.g.* Oxford Nanopore & PacBio).
- High Molecular Weight: DNA standard is >50 kb in size.
- Accurate Composition: Composition is cross-validated with multiple types of measurements.

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For Research Use Only

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

#### Product Contents

| Product Name                  | D6322         | Storage Temp. |
|-------------------------------|---------------|---------------|
| ZymoBIOMICS™ HMW DNA Standard | 5000 ng/50 µl | -20°C         |

Note: The DNA standard is shipped at ambient temperature which does not affect product quality. Recommended long term storage at -20°C upon receiving.

#### **Product Specifications**

- Source: seven bacteria (3 gram-negative and 4 gram-positive) and 1 yeast.
- Reference genomes and 16S&18S rRNA genes: <u>https://s3.amazonaws.com/zymo-files/BioPool/D6322.refseq.zip.</u>
- Storage solution: 10 mM Tris-HCl and 0.1 mM EDTA, pH 8.0.
- DNA concentration: 100 ng/µl.
- Impurity level: < 0.01% foreign microbial DNA.
- Average relative-abundance deviation: <15%.
- Microbial composition: Table 1 shows the theoretical microbial composition of the standard.

The microbial composition of each lot was measured by shotgun metagenomic sequencing post mixing. The results (including the composition, impurities and abundance deviation) can be accessed through the Certificate of Analysis based on the lot number (printed on tube level) by the following link: <a href="http://www.zymoresearch.com/microbiomics/microbial-standards/zymobiomics-microbial-community-standards">http://www.zymoresearch.com/microbiomics/microbial-standards/zymobiomics-microbial-community-standards</a>.

|                          | Theoretical Composition (%) |              |               |                             |                             |
|--------------------------|-----------------------------|--------------|---------------|-----------------------------|-----------------------------|
| Species                  | Genomic<br>DNA              | 16S<br>Only¹ | 16S &<br>18S¹ | Genome<br>Copy <sup>2</sup> | Cell<br>Number <sup>3</sup> |
| Pseudomonas aeruginosa   | 14                          | 5.1          | 4.6           | 7.8                         | 7.9                         |
| Escherichia coli         | 14                          | 12.4         | 11.2          | 10.9                        | 11.0                        |
| Salmonella enterica      | 14                          | 12.7         | 11.4          | 11.2                        | 11.2                        |
| Enterococcus faecalis    | 14                          | 12.1         | 10.9          | 18.8                        | 18.8                        |
| Staphylococcus aureus    | 14                          | 19           | 17.1          | 19.6                        | 19.6                        |
| Listeria monocytogenes   | 14                          | 17.3         | 15.6          | 17.8                        | 17.9                        |
| Bacillus subtilis        | 14                          | 21.4         | 19.2          | 13.2                        | 13.2                        |
| Saccharomyces cerevisiae | 2                           | NA           | 10            | 0.63                        | 0.32                        |

#### **Table 1: Microbial Composition**

<sup>1</sup> The theoretical composition in terms of 16S (or 16S & 18S) rRNA gene abundance was calculated from theoretical genomic DNA composition with the following formula: 16S/18S copy number = total genomic DNA (g) × unit conversion constant (bp/g) / genome size (bp) × 16S/18S copy number per genome. Use this as reference when performing 16S targeted sequencing.

 $^{2}$  The theoretical composition in terms of genome copy number was calculated from theoretical genomic DNA composition with the following formula: genome copy number = total genomic DNA (g) × unit conversion constant (bp/g) / genome size (bp). Use this as reference when inferring microbial abundance from shotgun sequencing data based on read depth.

<sup>3</sup> The theoretical composition in terms of cell number was calculated from theoretical genomic DNA composition with the following formula: cell number = total genomic DNA (g) × unit conversion constant (bp/g) / genome size (bp)/ploidy.

#### Notes:

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.

<sup>™</sup> Trademarks of Zymo Research Corporation. TapeStation<sup>®</sup> is a registered trademark of Agilent Technologies, Inc. MinION<sup>™</sup> is a trademark of Oxford Nanopore Technologies, Ltd.

#### Product Description

**ZymoBIOMICS<sup>™</sup> HMW DNA Standard** is a mixture of high molecular weight genomic DNA isolated from pure cultures of seven bacterial and one fungal strains (see Figure 1).<sup>1</sup> It has an accurately defined composition, negligible impurities (<0.01%) and contains genomes of a wide range of GC content (15%-85%). This can be used to assess and benchmark the performance of long read/3<sup>rd</sup> generation sequencing for microbiomics and metagenomics analysis and can also be used as a routine quality control (see Figure 2).

Details regarding the eight microbial strains (including species name, genome size, ploidy, average GC content, 16S/18S copy number, and phylogeny) can be found on page 4. The 16S/18S rRNA sequences (fasta format) and genomes (fasta format) of these strains are available at: <u>https://s3.amazonaws.com/zymo-files/BioPool/D6322.refseq.zip</u>. Feel free to contact us if we can help analyze the sequencing data generated from this standard.

**Background on the Need for Microbiome Standards:** 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 defined composition.



Figure 1. Genomic DNA from each species is >50 kb size. Sizing assessed by Agilent 2200 TapeStation<sup>®</sup> (Genomic DNA ScreenTape). The ZymoBIOMICS<sup>™</sup> HMW DNA Standard (STD) is composed of the following organisms: (PA) *Pseudomonas aeruginosa*, (EC) *Escherichia coli*, (SE) *Salmonella enterica*, (EF) *Enterococcus faecalis*, (SA) *Staphylococcus aureus*, (LM) *Listeria monocytogenes*, (BS) *Bacillus subtilis*, (SC) *Saccharomyces cerevisiae*.

#### Notes:

<sup>1</sup> Genomic DNA from each culture was extracted and quantified before mixing so this DNA standard was independent and not a direct derivative of the ZymoBIOMICS<sup>™</sup> Microbial Community Standard.

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A) Cumulative Throughput 1.2 M· 1 M 800 K Reads 600 K 400 K 200 K 0. 3h 20m 6h 40m 10h 13h 20m 16h 40m 20h 23h 20m 1d<sup>'</sup>2h 1d<sup>'</sup>6h 1d 9h 1d 12h Time **B)** Read Length Histogram 650 MB 600 MB 550 MB 500 MB Estimated Bases 450 MB 400 MB 350 MB 300 MB 250 MB -Total 200 MB 150 MB 100 MB 50 MB 0 B 📕 0 B 16.2 KB 32.4 KB 48.6 KB 64.8 KB 81 KB 97.2 KB 113.4 KB 129.6 KB Estimated Read Length in Bases **C)** Basecalling Quality Quality Score [4,20] = 40,000 - 30,000 - 20,000 20 10.00 (bases) [0,70000] 15.00 20,000 25,000 Read 30,000 Base 35,000 Figure 2. High Quality Long Read Sequencing. Long read sequencing was

Figure 2. High Quality Long Read Sequencing. Long read sequencing was performed on Oxford Nanopore MinION<sup>™</sup> using the Ligation Sequencing kit (LSK109) for library preparation. A) Cumulative throughput yield was 15.71 Gb (run for 36 hours). B) Read length histogram shows an approximate average of 24 kb with >125 kb recorded. C) Basecalling quality of number of reads shows overall good q-score of 11.

#### **Strain Information**

| Species                     | NRRL<br>Accession<br>NO. | Genome<br>Size (Mb) | Ploidy | GC<br>Content<br>(%) | 16/18S<br>Copy<br>Number | Gram<br>Stain |
|-----------------------------|--------------------------|---------------------|--------|----------------------|--------------------------|---------------|
| Pseudomonas<br>aeruginosa   | B-3509                   | 6.792               | 1      | 66.2                 | 4                        | -             |
| Escherichia coli            | B-1109                   | 4.875               | 1      | 46.7                 | 7                        | -             |
| Salmonella<br>enterica      | B-4212                   | 4.760               | 1      | 52.2                 | 7                        | -             |
| Enterococcus<br>faecalis    | B-537                    | 2.845               | 1      | 37.5                 | 4                        | +             |
| Staphylococcus<br>aureus    | B-41012                  | 2.730               | 1      | 32.9                 | 6                        | +             |
| Listeria<br>monocytogenes   | B-33116                  | 2.992               | 1      | 38.0                 | 6                        | +             |
| Bacillus subtilis           | B-354                    | 4.045               | 1      | 43.9                 | 10                       | +             |
| Saccharomyces<br>cerevisiae | Y-567                    | 12.1                | 2      | 38.3                 | 109 <sup>1</sup>         | Yeast         |

<sup>1</sup> 18S rRNA gene copy numbers in a haploid genome of the strain of *Saccharomyces cerevisiae* were estimated based on read depth information from mapping shotgun sequencing data.

Notes:

| Species                     | NCBI Phylogeny Database  |
|-----------------------------|--|
| Pseudomonas<br>aeruginosa   | Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;<br>Pseudomonadaceae; Pseudomonas; Pseudomonas aeruginosa group                                     |
| Escherichia coli            | Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;<br>Enterobacteriaceae; Escherichia   |
| Salmonella<br>enterica      | Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;<br>Enterobacteriaceae; Salmonella  |
| Enterococcus<br>faecalis    | Bacteria; Firmicutes; Bacilli; Lactobacillales; Enterococcaceae;<br>Enterococcus   |
| Staphylococcus<br>aureus    | Bacteria; Firmicutes; Bacilli; Bacillales; Staphylococcaceae;<br>Staphylococcus  |
| Listeria<br>monocytogenes   | Bacteria; Firmicutes; Bacilli; Bacillales; Listeriaceae; Listeria  |
| Bacillus subtilis           | Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus; Bacillus<br>subtilis group   |
| Saccharomyces<br>cerevisiae | Eukaryota; Opisthokonta; Fungi; Dikarya; Ascomycota; saccharomyceta;<br>Saccharomycotina; Saccharomycetes; Saccharomycetales;<br>Saccharomycetaceae; Saccharomyces |

#### **Protocol**

- 1. Thaw the standard on ice. After thawing, flick and spin down quickly.
- 2. The amount of DNA used depends on the library preparation process being evaluated.

### Appendix: Additional Strain Information

| Species                     | NRRL<br>Accession<br>NO. | Strain Name <sup>1</sup>   |
|-----------------------------|--------------------------|--|
| Bacillus subtilus           | B-354                    | Bacillus subtilis (Ehrenberg 1835) Cohn 1872 ATCC 6633=NRRL B-209=NRS-231=PCI 219  |
| Enterococcus<br>faecalis    | B-537                    | <i>Enterococcus faecalis</i> (Andrewes and Horder 1906) Schleifer and Kilpper-Bälz 1984 ATCC 7080  |
| Escherichia coli            | B-1109                   | Castellani and Chalmers 1919, 01485cm  |
| Listeria<br>monocytogenes   | B-33116                  | <i>Listeria monocytogenes</i> (Murray et al. 1926) Pirie 1940 2847=ATCC 19117  |
| Pseudomonas<br>aeruginosa   | B-3509                   | Pseudomonas aeruginosa (Schroeter 1872) Migula 1900<br>ATCC 15442=NCIB 10421=Pdd-10  |
| Saccharomyces<br>cerevisiae | Y-567                    | Saccharomyces cerevisiae Meyen ex E. C. Hansen<br>(1883) ATCC 9763=CBS 2978=CBS 5900=CCY 21-4-<br>48=CCY 21-4-54=NCTC 10716=NCTC 7239=NCYC<br>87=Pattee 6=PCI M-50 |
| Salmonella<br>enterica      | B-4212                   | <i>Salmonella enterica</i> subspecies <i>enterica</i> , Castellani and Chalmers 1919, TA1536   |
| Staphylococcus<br>aureus    | B-41012                  | Staphylococcus aureus Rosenbach 1884   |

<sup>1</sup> The strain information was extracted from the website of the Agricultural Research Service Culture Collection (NRRL, https://nrrl.ncaur.usda.gov/).

## **Ordering Information**

| Product Description                       | Size    | Catalog No. |
|---|---------|-------------|
| ZymoBIOMICS <sup>™</sup> HMW DNA Standard | 5000 ng | D6322       |

#### **Related Products**

| Related Products   | Size      | Catalog No. |
|--|-----------|-------------|
| ZymoBIOMICS <sup>™</sup> DNA Miniprep Kit  | 50 preps  | D4300       |
| ZymoBIOMICS <sup>™</sup> Microbial Community<br>Standard                           | 10 preps  | D6300       |
| ZymoBIOMICS <sup>™</sup> Microbial Community<br>DNA Standard (200ng)               | 200 ng    | D6305       |
| ZymoBIOMICS <sup>™</sup> Microbial Community<br>DNA Standard (2000ng)              | 2000 ng   | D6306       |
| ZymoBIOMICS <sup>™</sup> Microbial Community<br>Standard II (Log Distribution)     | 10 preps  | D6310       |
| ZymoBIOMICS <sup>™</sup> Microbial Community<br>DNA Standard II (Log Distribution) | 220 ng    | D6311       |
| ZymoBIOMICS <sup>™</sup> Spike-in Control I<br>(High Microbial Load)               | 25 preps  | D6320       |
| ZymoBIOMICS <sup>™</sup> Spike-in Control I<br>(High Microbial Load)               | 250 preps | D6320-10    |
| ZymoBIOMICS <sup>™</sup> Spike-in Control II<br>(Low Microbial Load)               | 25 preps  | D6321       |
| ZymoBIOMICS <sup>™</sup> Spike-in Control II<br>(Low Microbial Load)               | 250 preps | D6321-10    |

