ZYMO RESEARCH

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INSTRUCTION MANUAL

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

Catalog Nos. **D6320** & **D6320-10**

Highlights

- **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.

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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.

Product Contents

Product Name	D6320 (25 Preps)	D6320-10 (250 Preps)	Storage Temp.[1]
ZymoBIOMICS™ Spike-in Control I (High Microbial Load)	0.5 ml	0.5 ml x 10 tubes	- 80°C

[1] This product is shipped at room temperature, however please store at -80°C upon receipt.

Specifications

- **Biosafety:** this product is not biohazardous as microbes have been fully inactivated.
- **Complete reference genomes and 16S rRNA genes:** https://s3.amazonaws.com/zymo-files/BioPool/D6320.refseq.zip.
- **Storage solution:** DNA/RNA Shield™.
- **Impurity level:** < 0.01% foreign microbial DNA.
- **Microbial composition:** Table 1 shows the defined microbial composition of the standard.

Table 1: Microbial Composition

Species	Per Prep (20 µl)		
	Cells	16S Copies[1]	Total DNA[2] (ng)
Imtechella halotolerans	**2×10^7**	6.0×10^7	67.2
Allobacillus halotolerans	**2×10^7**	1.4×10^8	58.2

[1] 16S copies = cells × 16S copy number per cell/genome.
[2] Total genomic DNA (ng) = cells × genome size (bp/genome) × DNA unit conversion constant (ng/bp). DNA unit conversion constant (ng/bp) = 1.079×10^{-12}.

Table 2: Strain Information

Species	BCCM/LMG Accession NO.	Genome Size (Mb)	Ploidy	GC Content (%)	16S Copy Number	Gram Stain
Imtechella halotolerans	LMG 26483	3.113	1	35.6	3	-
Allobacillus halotolerans	LMG 24826	2.700	1	39.7	7	+

Table 3: Phylogeny and Strain Names

Acc. No.	LMG 26483	LMG 24826
Phylogeny	*Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Imtechella; Imtechella halotolerans*	*Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Allobacillus; Allobacillus halotolerans*
Strain Names[1]	JCM 17677; MTCC 11055; strain K1	BCRC 17939; Chen B3A

[1]The strain names were obtained from the website of the Belgian Co-ordinated Collections of Micro-organisms (BCCM, http://bccm.belspo.be/catalogues/lmg-catalogue-search).

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.

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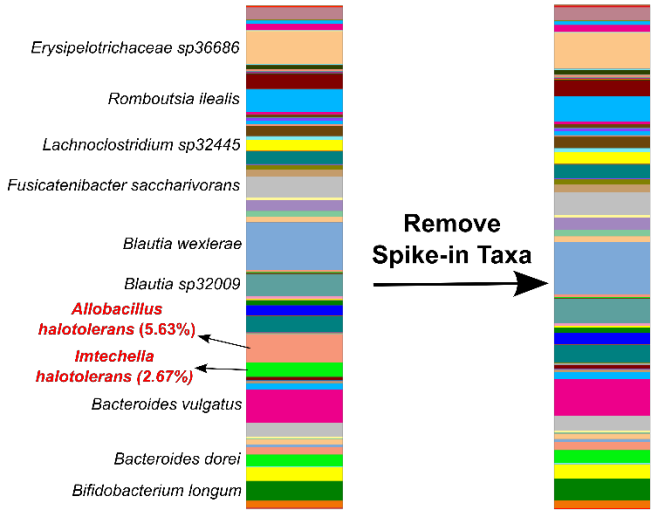
FastPrep™ is a trademark of MP Biomedicals.

Product Description

ZymoBIOMICS™ Spike-in Control I (High Microbial Load) consists of equal cell numbers of two bacteria strains, *Imtechella halotolerans and Allobacillus halotolerans*. When spiked into an unknown sample, this product will serve as an *in situ* positive control for DNA-sequencing-based (e.g. NGS-based) microbiome measurements. These two bacteria, *Imtechella halotolerans* (Gram-negative) and *Allobacillus halotolerans* (Gram-positive) represent different cell recalcitrance and can expose potential bias during DNA extraction. Moreover, with accurately quantified cell numbers, this standard enables absolute cell number quantification in microbiome measurements (as demonstrated in Figure 1).



To calculate the total cell number in this fecal sample (10 mg) from the abundance of *I. halotolerans*:

$$Total\ 16S\ copies = 16S\ copies\ of\ I.halotolerans \times \left(\frac{1}{Percentage\ of\ I.halotolerans} - 1\right)$$
$$= \frac{50\mu L}{20\mu L} \times 6.0 \times 10^7 \times \left(\frac{1}{2.67\%} - 1\right) = 5.47 \times 10^9$$

Assuming there are three 16S copies per genome:

$$Total\ cells \approx \frac{Total\ 16S\ copies}{3} = 1.8 \times 10^9$$

Similarly, the total cell number calculated from the abundance of *A. halotolerans* is 1.95 x 10^9

Figure 1. Quantify the total cell number in 10 mg of feces using the ZymoBIOMICS™ Spike-in Control I. 50 µl of ZymoBIOMICS™ Spike-in Control I was spiked into 100 µl of a fecal suspension, 10% (w/v) feces suspended in the solution of DNA/RNA Shield™. DNA extraction was performed with the ZymoBIOMICS™ DNA Miniprep Kit using 5 minutes of bead beating time on MP Fastprep™-24. 16S library was prepared with Quick-16S™ NGS Library Prep with primers targeting 16S V3-V4 region. Sequencing was performed on MiSeq™ using 600 cycles. Species-level taxonomy profiling was performed with the bioinformatics pipeline used by the ZymoBIOMICS™ 16S Service.

Protocol

1. Thaw the microbial standard completely on ice[1]. Then mix it thoroughly by vortexing.

   Cells may aggregate due to free-thaw cycling; therefore, it is critical to mix the standard thoroughly before use.

2. Exactly before DNA extraction, add the ZymoBIOMICS™ Spike-in Control I to your sample of interest at a final concentration of 0.1-10%. Then mix it thoroughly by vortexing. For feces, we recommend spiking-in 20 µl of the product into 10 mg of feces. Refer to Appendix A (page 3) about how to prepare 10 mg feces aliquots with DNA/RNA Shield™.

   20 µl of the standard contains 4 x 10^7 cells. For sample types with unknown cell concentration, the end-user might need to optimize the amount of the product to be added.

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

[1] Freeze-thaw cycling might reduce cell wall integrity but won't change the DNA content as the standard is preserved in DNA/RNA Shield®.

Sequencing Data Analysis

Be aware that microbial abundance can be presented in different ways depending on the bioinformatics tools or methods applied. For example, 16S sequencing analysis (*e.g.* using QIIME and Mothur) reports abundance by 16S copies. While shotgun pipelines that use marker genes rather than whole genomes as references (*e.g.* mOTU and Metaphlan2) likely report the abundance based on sequencing depth, which is equivalent to the abundance by genome copy number. Shotgun pipelines that use genomes as references (*e.g.* Centrifuge) likely report the abundance based on total quantity of reads assigned to genomes, which is more like the abundance by total genomic DNA mass. When comparing your results with the defined composition of the standard, remember to use the corresponding values shown in Table 1.

If the ratio between the abundance of two including bacteria (*I. halotolerans* and *A. halotolerans*) is quite different from the defined value (*e.g.* 3:7 by 16S copy), this might indicate potential bias in the workflow. For Example, if the abundance of *I. halotolerans* is much higher than that of *A. halotolerans*, this might indicate bias during DNA extraction because *A. halotolerans* is Gram-positive, tougher to lyse than *I. halotolerans*.

Appendix: How to Prepare 10 mg Feces with 20µl of the ZymoBIOMICS™ Spike-in Control I[1]

1. Add 1 ml of DNA/RNA Shield™ into a 1.5 ml microcentrifuge tube and weigh the tube (X mg).
2. Add 15-100 mg of feces into the tube DNA/RNA Shield™ and weight it again (Y mg).
3. Completely re-suspend the feces by vortexing.
4. Determine the final feces concentration of the suspension as (Y-X)/1000 mg/µl.
5. Based on the concentration, withdraw a volume of the fecal suspension that is equivalent to 10 mg of feces and add it into a ZR BashingBead™ lysis tube (part of ZymoBIOMICS™ DNA Miniprep Kit).
6. Vortex the ZymoBIOMICS Spike-in Control I thoroughly and add 20 µl of the ZymoBIOMICS Spike-in Control I into the ZR BashingBead™ lysis tube and adjust the final volume to 1000 µl with DNA/RNA Shield™.
7. Proceed to mechanical lysis by following the protocol of ZymoBIOMICS™ DNA Miniprep Kit.

[1] In this protocol, DNA/RNA Shield™ and ZR BashingBead™ lysis tube scan be replaced by corresponding cell lysis solution and mechanical bead tubes depending on the DNA extraction kit you use.

Ordering Information

Product Description	Size	Catalog No.
ZymoBIOMICS™ Spike-in Control I (High Microbial Load)	25 preps	D6320
ZymoBIOMICS™ Spike-in Control I (High Microbial Load) – 250 preps	250 preps	D6320-10

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

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