INSTRUCTION MANUAL

ZymoBIOMICS™ Spike-in Control II (Low Microbial Load)

Catalog Nos. **D6321** & **D6321-10**

Highlights

- **Absolute Quantification:** Enables cell number measurements from low bacterial load samples using Next-Gen Sequencing.
- ***In situ* Quality Control:** Ensures each sample is quantified accurately.
- **Log Abundance Distribution:** Three microbes alien to the human microbiome in log-distribution from 10^3 to 10^5 cells

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.

Product Contents:

Product Name	D6321 (25 Preps)	D6321-10 (250 Preps)	Storage Temperature
ZymoBIOMICS™ Spike-in Control II (Low Microbial Load)	0.5 ml	0.5 ml x 10	- 80°C

Note - Integrity of product components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide maximal performance and reliability.

Specifications:

- **Biosafety:** this product is not biohazardous as microbes have been fully inactivated.
- **Reference genomes and 16S rRNA genes:** https://s3.amazonaws.com/zymo-files/BioPool/D6321.refseq.zip
- **Storage solution:** DNA/RNA Shield™
- **Impurity level:** <0.01% foreign microbial DNA
- **Microbial composition:** Refer to Table 1 for the theoretical composition of the standard and Table 2 for the strain information.

Table 1: Microbial Composition

Species	Per Prep (20 µL)		
	Cells	16S copies[1]	Total DNA[2] (ng)
Truepera radiovictrix	1.0 x 10^5	2.0 x 10^5	0.35
Imtechella halotolerans	1.0 x 10^4	3.0 x 10^4	0.034
Allobacillus halotolerans	1.0 x 10^3	7.0 x 10^3	0.0029

[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 x 10^{-12}

Table 2: Strain Information

Species	BCCM/LMG Accession Number	Genome Size (Mb)	Ploidy	GC Content (%)	16S Copy Number	Gram Stain
T. radiovictrix	LMG 22925	3.26	1	68.1	2	n/a[3]
I. halotolerans	LMG 26483	3.11	1	35.6	3	-
A. halotolerans	LMG 24826	2.70	1	39.7	7	+

[3] The gram stain for *Truepera radiovictrix* is unable to be determined.

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.

Product Description:

ZymoBIOMICS™ Spike-in Control II (Low Microbial Load) consists of three bacteria strains, *Truepera radiovictrix*, *Imtechella halotolerans*, and *Allobacillus halotolerans*. When spiked into a microbial sample, this product will serve as an *in situ* positive control for DNA-sequencing-based microbiome measurements. *Imtechella halotolerans* is Gram-negative and *Allobacillus halotolerans* is Gram-positive while, *Truepera radiovictrix* is resistant to lysozyme lysis and has very high GC content. These species represent different challenges in NGS-based analysis. Moreover, with accurately quantified cell number and a log abundance distribution, this standard enables absolute cell number quantification in cases such as pathogen load detection.

Accurately Determine Pathogen Load in Clinical Samples

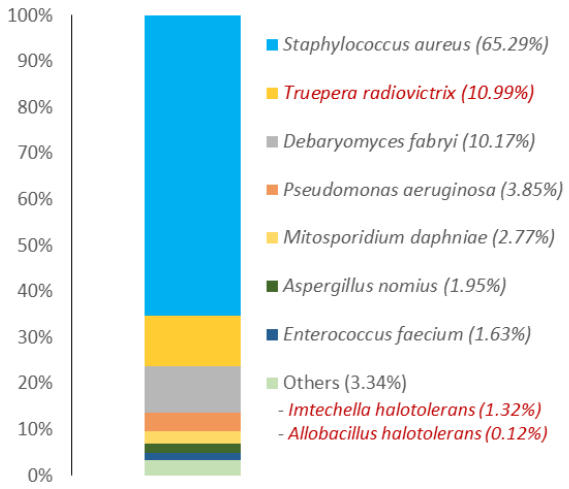

Figure 1. ZymoBIOMICS™ Spike-in Control II can be used to quantify pathogens in clinical samples. 20 µl of ZymoBIOMICS™ Spike-in Control II was spiked into 200 µl of a sputum sample. DNA extraction was performed with the ZymoBIOMICS™ DNA Miniprep Kit. The Shotgun library was generated using the Kapa HyperPlus™ kit and sequencing was performed on HiSeq® with 2x100 bp. Strain-level taxonomy profiling was performed with Centrifuge and the microbial abundance was inferred from the read counts assigned.

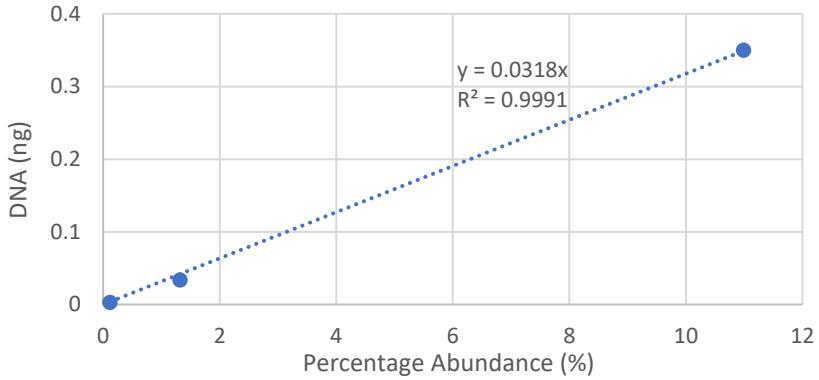

Figure 2. Absolute abundance quantification. The relative abundances of the three spike-in strains are used to draw a standard curve for quantification of other microbial organisms

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

Calculating Potential Pathogen Loads

1. Using the defined total DNA abundance (ng) of the three spike-in microbes in Figure 1, a standard curve was generated (Figure 2). The standard curve equation is $y = 0.0318x$. Therefore 1% of abundance is equivalent to 0.0318 ng of DNA in this case.

2. Taking *S. aureus* as an example, the relative abundance from sequencing was equal to 65.29%. The total DNA can be calculated as:

$$\text{DNA abundance} = 65.29 \times 0.0318 = 2.08ng$$

3. The absolute abundance cell number for *S. aureus* can then be calculated as:

$$\text{Cell number} = \frac{2.08ng}{(2.8\times10^{6}\ bp/cell)\times(1.079\times10^{-12}ng/bp)} = 6.9 \times 10^{5}\ \text{cells}$$

4. Similarly, the abundances for all other microbes in the sample can be calculated as shown in the table below:

Table 3: Potential Pathogen Absolute Abundances

	Microbe	DNA (%)	DNA (ng)	Genome Size (Mb)	Cells
ZymoBIOMICS™ Spike-in Control II	*T. radiovictrix*	11.0	0.35	3.26	9.94×10^4
	I. halotolerans	1.32	0.034	3.11	1.01×10^4
	A. halotolerans	0.12	0.0029	2.7	9.95×10^2
Potential pathogens	*S. aureus*	65.3	2.08	2.8	6.9×10^5
	P. aeruginosa	3.85	0.12	5.9	1.9×10^4
	E. faecium	1.63	0.052	2.6	1.8×10^4
	herpesvirus 4 type 2	0.12	0.0038	0.155	2.3×10^4

Protocol:

Sample Preparation

1. Thaw the microbial standard completely on ice. Mix thoroughly by vortex[1].

2. Exactly before DNA extraction, spike 20 µl of the ZymoBIOMICS™ Spike-in Control II into your sample of interest and vortex to mix well. The amount of the standard to spike in will vary depending on the sample type.

20 µl of the standard contains 1.11 x 10^5 cells. Ideally, the spike-in microbial abundance will be between 0.1% - 10%. For a specific sample type, this needs to be optimized by the end-user.

Notes:

[1] It is critical to mix the standard thoroughly before use to avoid cell clumping. End quantification will be inaccurate if the cells are not evenly suspended.

Sequencing Data Analysis

Microbial abundance can be presented in different ways depending on the bioinformatics tools or methods applied.

Targeted 16S sequencing analysis (*e.g.* using QIIME and Mothur) reports abundance by 16S copies. Many shotgun pipelines that use marker genes rather than whole genomes as references (*e.g.* mOTUs and Metaphlan2) 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 similar to 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 abundance of including bacteria in the spike-in standard is quite different from their defined values (*Table 1*), this might indicate potential bias in the workflow.

Absolute Abundance Quantification Calculation

Use the defined DNA abundance (ng) of the three spike-in microbes to draw a standard curve. The standard curve equation should be in the format $y = ax$, where a represents the ng of DNA per 1% abundance.

Calculate the DNA abundance of your sample organism of interest using the standard curve.

The absolute cell number can then be calculated as:

$$\text{Cell number} = \frac{\text{ng of DNA}}{(\text{genome size}) \times (\text{DNA unit conversion constant})}$$

where the DNA unit conversion constant is equivalent to $1.079 \times 10^{-12}\, ng/bp$.

Appendix: Phylogeny and Strain Information

Acc. No.	LMG 22925	LMG 26483	LMG 24826
Phylogeny	*Bacteria; Deinococcus-Thermus; Deinococci; Deinococcales; Trueperaceae; Truepera; Treupera radiovictrix*	*Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Imtechella; Imtechella halotolerans*	*Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Allobacillus; Allobacillus halotolerans*
Strain Names[1]	CIP 108686; da Costa RQ-24; DSM 17093	JCM 17677; MTCC 11055; strain K1	BCRC 17939; Chen B3A

[1] The strain names were extracted from the website of the Belgian Coordinated Collections of Microorganisms (BCCM, http://bccm.belspo.be/catalogues/lmg-catalogue-search).

Ordering Information:

Product Description	Kit Size	Catalog No.
ZymoBIOMICS™ Spike-in Control II (Low Microbial Load)	25 Preps 250 Preps	D6321 D6321-10

Related Products

Product Description	Kit Size	Catalog No.
ZymoBIOMICS™ DNA Miniprep Kit	5 Preps 50 Preps 50 Preps	D4300T D4300 D4303
ZymoBIOMICS™ Microbial Community Standard	10 Preps	D6300
ZymoBIOMICS™ Microbial Community DNA Standard (200ng)	200 ng / 20 µl	D6305
ZymoBIOMICS™ Microbial Community DNA Standard (2000ng)	2000 ng / 20 µl	D6306
ZymoBIOMICS™ Microbial Community Standard II (Log Distribution)	10 Preps	D6310
ZymoBIOMICS™ Microbial Community DNA Standard II (Log Distribution)	220 ng / 20 µl	D6311
ZymoBIOMICS™ Spike-in Control I (High Microbial Load)	25 Preps 250 Preps	D6320 D6320-10

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