INSTRUCTION MANUAL

HostZERO™ Microbial DNA Kit

Catalog No. **D4310**

Highlights

- **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.

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Product Contents:

HostZERO™ Microbial DNA Kit (Kit Size)	D4310 (50 Preps.)	Storage Temperature
Host Depletion Solution[1]	3 x 20 ml	-20°C
Microbial Selection Buffer	5 ml	-20°C
Microbial Selection Enzyme	50 µl	-20°C
Proteinase K & Storage Buffer[2]	20 mg	-20°C
DNA/RNA Shield™ (2X Concentrate)	5 ml	Room Temp.
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	50	Room Temp.
ZymoBIOMICS® Lysis Solution	40 ml	Room Temp.
ZymoBIOMICS® DNA Binding Buffer	100 ml	Room Temp.
ZymoBIOMICS® DNA Wash Buffer 1	50 ml	Room Temp.
ZymoBIOMICS® DNA Wash Buffer 2	60 ml	Room Temp.
ZymoBIOMICS® DNase/RNase Free Water	3 ml	Room Temp.
Zymo-Spin™ IC-Z Columns	50	Room Temp.
Collection Tubes	200	Room Temp.
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Note - Integrity of kit components are 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.

[1] For optimal performance, minimize freeze-thaws of Host Depletion Solution by making smaller aliquots.

[2] Prior to use, reconstitute Proteinase K with 1,040 µl of the Proteinase K Storage Buffer. Store at -20°C after mixing.

Specifications:

- **Sample Input:** Up to 200 µl liquid sample.
- **Sample Type:** Samples such as saliva, swabs, and bodily fluids from eukaryotic hosts with intact bacteria cells are compatible with this kit. Samples that have undergone freeze-thaw cycles or that have been stored in solution that affect the integrity of the bacterial cells will experience loss of bacterial DNA. Not compatible with tissue or blood samples or samples stored in DNA/RNA Shield™.
- **DNA Recovery:** Greater than 85% of total bacterial DNA is effectively recovered with greater than 90% of eukaryotic host DNA depleted.
- **DNA Purity:** High-quality DNA is eluted with ZymoBIOMICS® DNase/RNase Free Water and is suitable for all downstream applications including PCR and Next-Generation Sequencing.
- **Required Equipment:** Microcentrifuge, vortex, tube rotator, and high-speed cell disruptor (recommended).
- **Processing Time:** Bacterial DNA is isolated from a single sample in less than 90 minutes.

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:

The **HostZERO™ Microbial DNA Kit** is designed to overcome the challenge of contaminating host nucleic acids in microbial samples. This kit uses a novel method to reduce the amount of contaminating host DNA by selectively lysing the eukaryotic cells and degrading this DNA prior to total DNA purification. Paired with Zymo Research's non-biased purification technology, the HostZERO™ Microbial DNA Kit allows for the exclusive capture of DNA from living microbial cells in a biological sample. This new technology is able to reduce the presence of human DNA in a saliva sample from 65% (untreated sample) to less than 1% (treated sample, Figure 1). Concurrently, the depletion process utilizes the ZymoBIOMICS® non-biased DNA isolation technology for accurate data representation (Figure 2). Finally, the HostZERO™ Microbial DNA Kit recovers the highest amount of bacterial DNA as compared to other methods (Figure 3). By removing the presence of host DNA and reducing bias in purification, the HostZERO™ Microbial DNA Kit produces the highest-quality data for microbial samples.

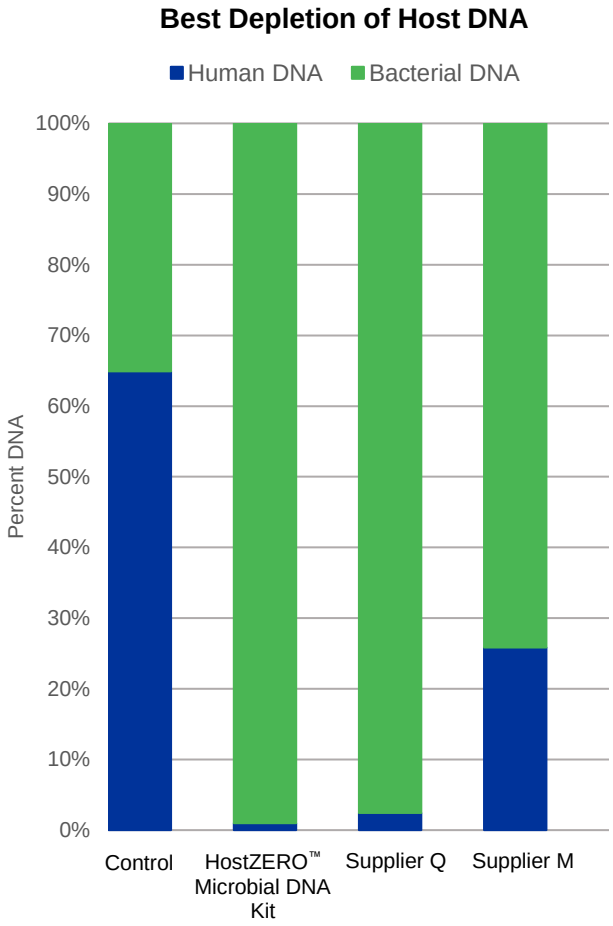

Figure 1. The HostZERO™ Microbial DNA Kit depletes the greatest amount of human DNA.

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

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

Product Description (Continued):

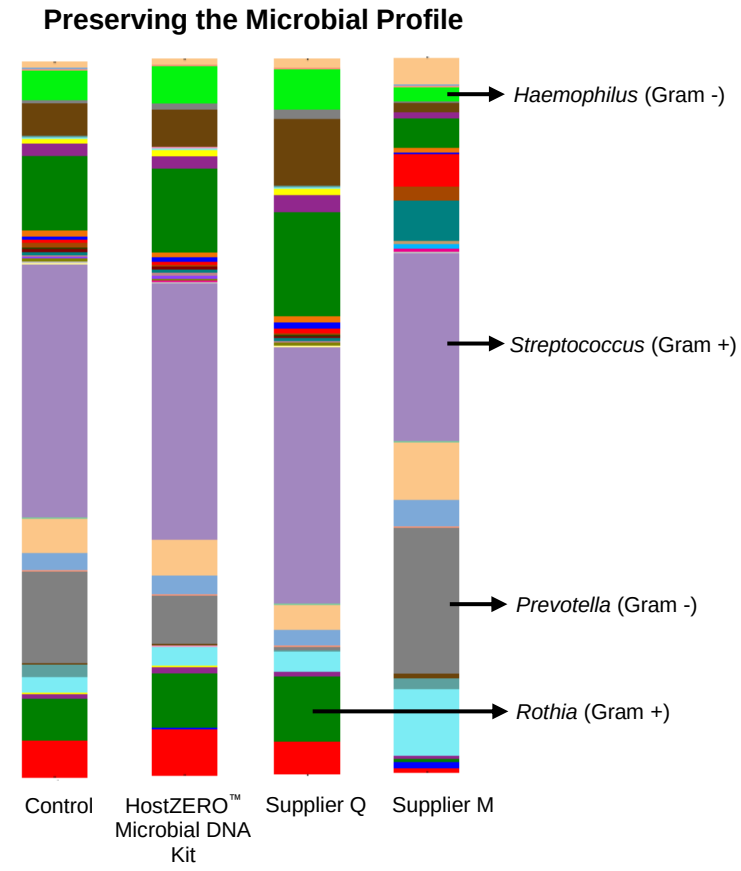

Figure 2. The microbial composition is maintained best in samples treated with the HostZERO™ Microbial DNA Kit.

The same human saliva sample was processed using the HostZERO™ Microbial DNA Kit or kits from Suppliers Q and M. Purified DNA was analyzed using 16S rRNA gene targeted sequencing. Primers targeting the 16S V3-V4 region were used, and the amplicons were sequenced on the Illumina MiSeq® (2x300bp). The control method is the same sample processed with the ZymoBIOMICS® DNA Microprep Kit, which extracts total DNA from the sample without host DNA depletion. Samples were processed in triplicates.

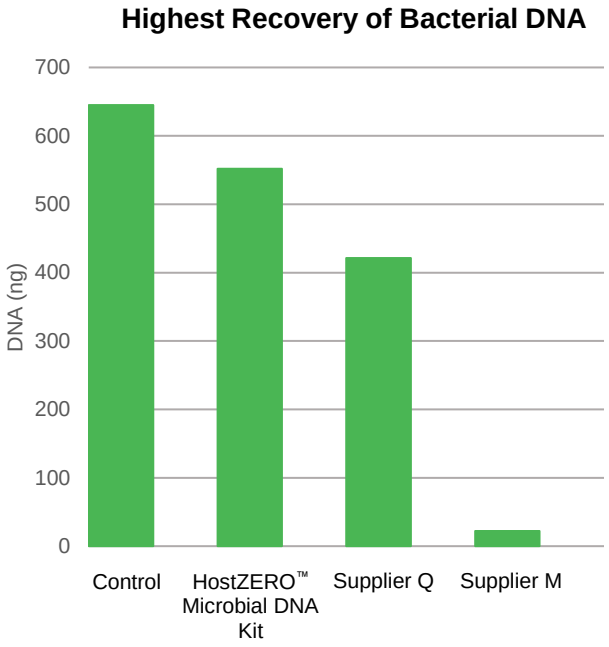

Figure 3. Bacterial DNA is effectively recovered with HostZERO™ technology.

The same human saliva sample was processed using the HostZERO™ Microbial DNA Kit or kits from Suppliers Q and M. Real-time PCR was used to evaluate purified DNA. The control method is the same sample processed with the ZymoBIOMICS® DNA Microprep Kit, which extracts total DNA from the sample without host DNA depletion. Samples were processed in triplicates.

Procedure Overview:

Step 1
Depletion of eukaryotic host DNA from sample.

Add Host Depletion Solution directly to sample.

Step 2
Unbiased lysis of remaining bacterial cells.

Lyse sample with ZR BashingBead™ Lysis Tube (0.1 mm & 0.5 mm).

Step 3
Isolation of bacterial DNA.

Bind, wash, and elute DNA with Zymo-Spin™ IC-Z.

Isolated Bacterial DNA

Notes:

Reagent Preparation:

✓ Add 1,040 µl of **Proteinase K Storage Buffer** to the **Proteinase K (20 mg)** tube and mix thoroughly prior to use. The final concentration of **Proteinase K** is ~20 mg/ml. Store at -20°C after mixing.

Before Starting:

✓ Preheat heating blocks or water baths to 37°C and 55°C.

Protocol:

The following procedure should be performed at room temperature (15-30°C) unless specified.

Section 1: Host DNA Depletion

1. In a 1.5 ml microcentrifuge tube (not provided), add 1 ml **Host Depletion Solution** to 200 µl of sample[1,2].
2. Rotate sample for 15 minutes using end-over-end rotation at room temperature.
3. Centrifuge the tube in a microcentrifuge at 10,000 x *g* for 5 minutes.
4. Without disturbing the pellet, carefully remove and discard the supernatant.
5. Add 100 µl of **Microbial Selection Buffer** to the tube and resuspend the pellet.
6. Add 1 µl of **Microbial Selection Enzyme** to the suspension. Vortex briefly to mix.
7. Incubate the tube at 37°C for 30 minutes.
8. Proteinase K Treatment (Recommended)

   Add 20 µl of **Proteinase K** to the sample and vortex for at least 10 seconds. Incubate at 55°C for 10 minutes[3].

   Treatment with Proteinase K enhances depletion of host DNA; however, at longer incubation times, it can also result in the loss of bacterial DNA in species sensitive to enzymatic digestion.
9. Add 100 µl of **DNA/RNA Shield™ (2X Concentrate)** to the sample and vortex for at least 10 seconds. Incubate at room temperature for 5 minutes.
10. Proceed to Section 2: Microbial DNA Isolation or store sample at -80ºC.

[1] The volume of Host Depletion Solution can be scaled up or down according to sample input volume.

[2] Swabs should be cut to fit directly in the microcentrifuge tube. For more information on processing swab samples, see Appendix A.

[3] The duration of the incubation can be optimized by sample type to achieve maximum depletion of host DNA with minimum loss of bacterial DNA. The total incubation time should not exceed 30 minutes.

Section 2: Microbial DNA Isolation

1. Add the entire sample from the end of Section 1 to a **ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)**. Add 750 µl of **ZymoBIOMICS® Lysis Solution** to the tube and cap tightly.

2. Secure in a bead beater fitted with a 2 ml tube holder assembly and process at maximum speed for 3 minutes[1,2].

3. Centrifuge the ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm) at ≥ 10,000 x *g* for 1 minute.

4. Transfer 400 µl of supernatant to a **Collection Tube**.

5. Add 1,200 µl of **ZymoBIOMICS® DNA Binding Buffer** to the supernatant in the Collection Tube. Mix thoroughly by pipetting the entire volume up and down five times.

6. Place a **Zymo-Spin™ IC-Z Column** in a new Collection Tube. Transfer 800 µl of the mixture from Step 5 to the Zymo-Spin™ IC-Z Column. Centrifuge at 10,000 x *g* for 1 minute.

7. Discard the flow through from the Collection Tube and repeat Step 6.

8. Add 400 µl of **ZymoBIOMICS® DNA Wash Buffer 1** to the Zymo-Spin™ IC-Z Column in a new Collection Tube. Centrifuge at 10,000 x *g* for 1 minute, then discard the flow through.

9. Add 700 µl of **ZymoBIOMICS® DNA Wash Buffer 2** to the Zymo-Spin™ IC-Z Column in the same Collection Tube. Centrifuge at 10,000 x *g* for 1 minute, then discard the flow through.

10. Add 200 µl of ZymoBIOMICS® DNA Wash Buffer 2 to the Zymo-Spin™ IC-Z Column in the same Collection Tube. Centrifuge at 10,000 x *g* for 1 minute, then discard the Collection Tube.

11. Transfer the Zymo-Spin™ IC-Z Column to a new 1.5 ml microcentrifuge tube (not provided). Add 20 µl of **ZymoBIOMICS® DNase/RNase Free Water** directly to the column matrix and incubate at room temperature for 5 minutes. Centrifuge at 10,000 x *g* for 1 minute to elute the DNA.

Notes:

[1] Processing time will vary based on sample input and bead beater. Times may be as little as 3 minutes when using high-speed cell disrupters (e.g. FastPrep®-24) or as long as 40 minutes when using lower speeds (e.g. Disruptor Genie®).

[2] For optimal lysis efficiency and unbiased profiling, all bead beater devices beyond those validated by Zymo Research should be calibrated using the ZymoBIOMICS® Microbial Community Standard. For more information, see Appendix B.

Appendix A: Samples Collected with Swabs

Place swab directly into a 1.5 ml microcentrifuge tube (not provided) with 1 ml of Host Depletion Solution. If there is liquid associated with the swab, transfer 200 µl of sample and the swab into the tube. The swab should be cut at the height of the microcentrifuge tube and left inside during Steps 2 and 3 (Page 5). Carefully remove the swab from the tube after centrifugation in Step 3 (Page 5). Use a sterile method to cut the swab and to remove it from the 1.5 ml microcentrifuge tube to prevent the introduction of external sources of microbes or DNA.

Appendix B: Microbial Composition of the ZymoBIOMICS® Microbial Community Standard

The **ZymoBIOMICS® Microbial Community Standard (Cat. No. D6300)** is a mock microbial community of defined and well-characterized composition, making it the perfect control for all microbiome profiling and metagenomics analyses.

The standard can be used to validate DNA isolation using bead beater devices to ensure that an unbiased, representative profile of the microbial samples is achieved during lysis. Serving as a defined input from the beginning, this standard can be used to guide construction and optimization of entire workflows and as a quality control for inter-lab studies. Benchmarking with this standard, Zymo Research has found that most cited DNA extraction methods are significantly biased (Figure 4).

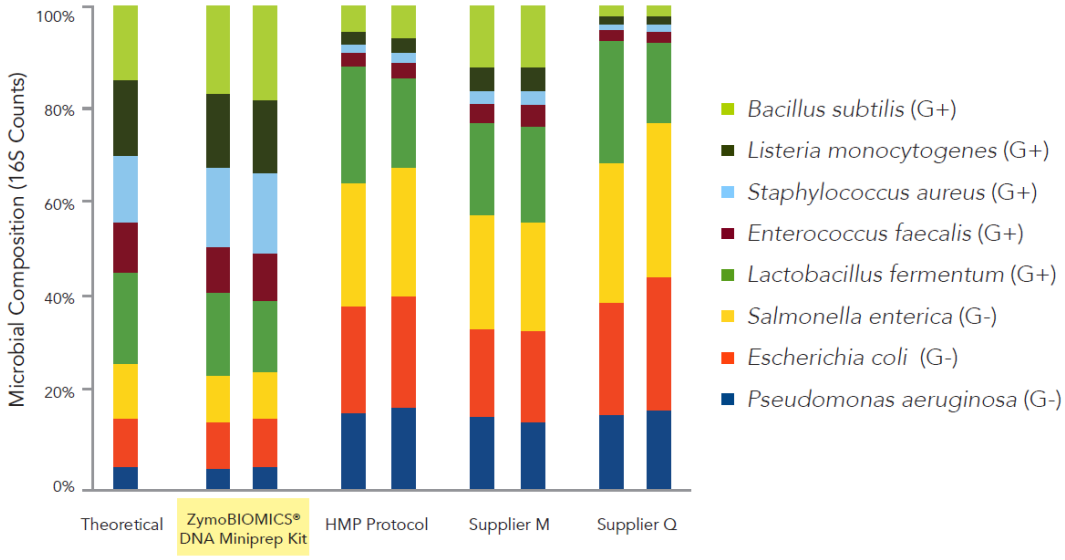

Figure 4. Benchmarking DNA extraction processes with ZymoBIOMICS® Microbial Community Standard.

DNA was extracted from ZymoBIOMICS® Microbial Community Standard using the four different DNA extraction methods (ZymoBIOMICS® DNA Miniprep Kit, Human Microbiome Project fecal DNA extraction protocol, a DNA extraction kit from Supplier M, or a fecal DNA extraction kit from Supplier Q) and analyzed using 16S rRNA gene sequencing. 16S rRNA genes were amplified with primers targeting V3-V4 region and the amplicons were sequenced on Illumina® MiSeq® (2x250bp). Overlapping paired-end reads were assembled into complete amplicon sequences. The composition profile was determined based on sequence counts after mapping amplicon sequences to the known 16S rRNA genes of the eight different bacterial species contained in the standard. Only the ZymoBIOMICS® DNA Miniprep Kit provides unbiased profiles in this comparison.

Troubleshooting Guide:

Problem	Possible Causes and Suggested Solutions
Low Bacterial DNA Yield	
Proteinase K incubation is too long	• **Ensure that the Proteinase K incubation does not exceed 30 minutes.** Some bacterial species are sensitive to degradation by Proteinase K and will become lysed during the incubation. The duration of the incubation should be optimized by sample type for sufficient depletion of host DNA and recovery of bacterial DNA.
DNA/RNA Shield™ incubation is too short	• **Ensure that DNA/RNA Shield™ incubation is at least 5 minutes.** DNA/RNA Shield™ is thoroughly mixed with the sample and incubated with the sample for at least 5 minutes before proceeding to Section 2: Microbial DNA Isolation.
Incomplete lysis	• **For tough-to-lyse bacteria, ensure bead beating duration and speed are optimized to the device.** For optimal performance, use the ZymoBIOMICS® Microbial Community Standard (Zymo Research, Catalog No. D6300) to determine the best processing time and speed.
Excessive lysis	• **For easy-to-lyse bacteria, optimize bead beating duration and speed to prevent over-shearing the bacterial DNA.** For best results, optimize the bead beating system by testing the lysis of easy-to-lyse bacteria, beginning with shorter times and lower speeds.
Binding buffer not mixed well with sample	• **Ensure that the ZymoBIOMICS® DNA Binding Buffer is completely mixed with lysate before loading onto the column.** Improperly mixed samples can lead to poor DNA recovery.
DNA elution	• **Ensure that the ZymoBIOMICS® DNase/RNase Free Water hydrates the column matrix for at least 5 minutes before centrifugation.** Shortening this time may reduce DNA yields. • **To increase yields, the ZymoBIOMICS® DNase/RNase Free Water can be heated to 60°C before use.** Additionally, the eluate can be reloaded onto the column matrix, incubated at room temperature for 3 minutes, and centrifuged again to increase yield.
High Host DNA Yield	
Insufficient incubation	• **Ensure that the incubations during Section 1: Host DNA Depletion are performed at the proper temperatures and for the full amount of time.** Reducing the incubation times can result in an excess amount of host DNA.
Insufficient Proteinase K treatment	• **Ensure that the Proteinase K incubation time is long enough to sufficiently deplete remaining host DNA.** The length of the incubation should be optimized by sample type to determine the optimal time for sufficient depletion of host DNA and recovery of bacteria DNA. Treatment with Proteinase K enhances depletion of host DNA; however, at longer incubation times, it can also result in the loss of bacterial DNA in species sensitive to enzymatic digestion.
Background Contamination	
Workspace contamination	• **Clean workspace, centrifuge, and pipettes with 10% bleach followed by 70% ethanol routinely to avoid contamination.** • **Use of kit in an exposed environment can lead to background contamination.** Check pipettes, pipettes tip, microcentrifuge tubes, workspace, etc. for contamination. Zymo Research recommends working in a clean room or enclosed workspace. • **Make sure bags of columns and reagent bottles are properly sealed for storage.** Use of these outside a clean room or hood can result in contamination.

Ordering Information:

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

For Individual Sale	Amount	Catalog No.
Host Depletion Solution	20 ml	D4310-1-20
Microbial Selection Buffer	5 ml	D4310-2-5
Microbial Selection Enzyme	50 µl	D4310-3-50
Proteinase K & Storage Buffer	5 mg 20 mg	D3001-2-5 D3001-2-20
DNA/RNA Shield™ (2X Concentrate)	25 ml 125 ml	R1200-25 R1200-125
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	50	S6012-50
ZymoBIOMICS® Lysis Solution	40 ml	D4300-1-40
ZymoBIOMICS® DNA Binding Buffer	100 ml	D4300-2-100
ZymoBIOMICS® DNA Wash Buffer 1	50 ml	D4300-3-50
ZymoBIOMICS® DNA Wash Buffer 2	60 ml	D4300-4-60
ZymoBIOMICS® DNase/RNase Free Water	10 ml 50 ml	D4302-5-10 D4302-5-50
Collection Tubes	50 500 1000	C1001-50 C1001-500 C1001-1000


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