ZYMO RESEARCH

Microbiomics
Made Simple™

HostZERO™ Microbial DNA Kit

Streamlined host depletion and bacterial DNA isolation from mammalian samples

Highlights

- ≥90% host DNA depletion in applicable sample types.
- Only 30 minutes of hands-on time for purification of high-quality DNA.
- Unbiased cellular lysis for accurate analysis of the microbial profile.

Catalog Number:
D4310

Scan with your smart-phone camera to view the online protocol/video.

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682

Table of Contents

Product Contents

HostZERO™ Microbial DNA Kit	D4310 (50 preps.)	Storage Temperature
Host Depletion Solution[1]	20 ml (x3)	-20°C
Microbial Selection Buffer	5 ml	-20°C
Microbial Selection Enzyme	50 µl	-20°C
Proteinase K[2] (lyophilized) **& Storage Buffer**	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.
Instruction Manual	1 pc	-

1 For optimal performance, minimize freeze-thaws of Host Depletion Solution by making smaller aliquots.
2 Add Proteinase K Storage Buffer to the lyophilized Proteinase K, see Buffer Preparation, page 5. Store frozen aliquots.

Specifications

- **Sample Input –** Up to 200 µl of liquid sample[1].
- **Sample Sources –** Samples such as saliva, swabs, and bodily fluids from eukaryotic hosts with intact bacteria cells are directly compatible with this kit. Pre-treatment is required for whole blood[2] and solid tissue[2] samples. Samples that have undergone multiple 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 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.
- **Equipment Needed –** Microcentrifuge, Vortex/Disruptor Genie®, high speed cell disrupter (recommended).

1 See Appendix B for details on processing liquid samples with higher volumes.
2 See Appendix B for details on processing whole blood and tissue samples.

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.

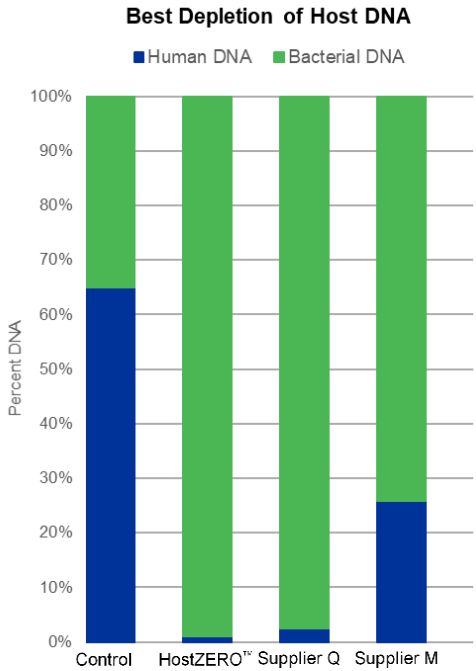


Figure 1. The HostZERO™ Microbial DNA Kit depletes the greatest amount of human DNA. The same human saliva sample was processed using the HostZERO™ Microbial DNA Kit or kits from Suppliers Q and M. Real-time PCR was used to evaluate purified DNA. The composition of the DNA is shown in terms of bacterial 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.

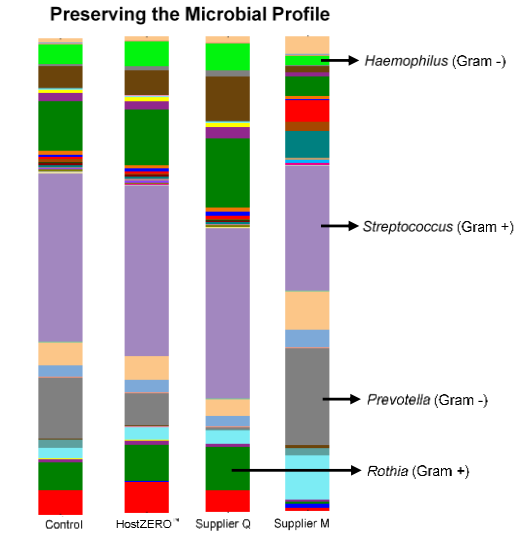


Figure 2. The microbial composition is maintained best in samples treated with the HostZERO™ Microbial DNA Kit. The same human saliva sample was processed using the HostZERO™ Microbial DNA Kit or kits from Suppliers Q and M. Purified DNA was analyzed using 16S rRNA gene targeted sequencing. Primers targeting 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.

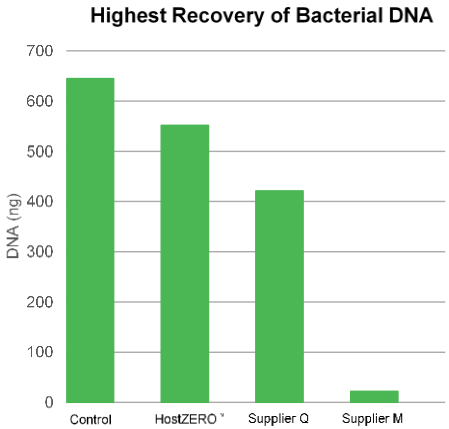


Figure 3. Bacterial DNA is effectively recovered with HostZERO™ technology. The same human saliva sample was processed using the HostZERO™ Microbial DNA Kit or kits from Suppliers Q and M. Real-time PCR was used to evaluate purified DNA. The control method is the same sample processed with the ZymoBIOMICS™ DNA Microprep Kit, which extracts total DNA from the sample without host DNA depletion. Samples were processed in triplicates.

Purification Guide

Step 1
Depletion of eukaryotic host DNA from sample.

Add Host Depletion Solution directly to sample.

Step 2
Unbiased lysis of remaining microbial cells.

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

Step 3
Isolation of microbial DNA.

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

Ultra-Pure Microbial DNA

Protocol

The protocol consists of (I) Buffer Preparation, (II) Host DNA Depletion, and (III) Microbial DNA Isolation.

Before starting: Preheat heating block or water baths to 37°C and 55°C.

(I) Buffer Preparation

- ✓ Reconstitute lyophilized **Proteinase K** at 20 mg/ml with **Proteinase K Storage Buffer** and mix by vortexing. Use immediately or store frozen aliquots:
  #D3001-2-20 (20 mg), add 1.04 ml **buffer**
  #D3001-2-5 (5 mg), add 0.26 ml **buffer**

(II) Host DNA Depletion

1. In a clean microcentrifuge tube, add 1 ml **Host DNA Depletion Solution** to 200 µl of sample[1,2,3].

2. Rotate sample for 15 minutes using end-over-end rotation at room temperature (20-30ºC).

3. Centrifuge the tube 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.

1 The volume of Host Depletion Solution can be scaled up or down according to sample input volume. See Appendix B for more information.
2 Swabs should be cut to fit directly in the microcentrifuge tube. For more information on processing swab samples, see Appendix A.
3 For processing whole blood, sputum, and tissue samples, see Appendix B.

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.[1,2]

9. Add 100 µl of **DNA/RNA Shield™ (2X Concentrate)** to the sample and vortex for at least 10 seconds. Incubate at room temperature (20-30ºC) for 5 minutes.

10. Proceed to (III) Microbial DNA isolation or store sample at -80°C.

(III) Microbial DNA Isolation

- ✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 *x g* for 30 seconds, unless specified.

1. Add the entire sample from the end of (II) Host DNA Depletion 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 5 minutes[3].

Note: Processing time will vary based on sample input and bead beater. For validated bead beating devices and conditions, refer to the Optimized Lysis Protocols (Appendix D).

3. Centrifuge the ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm) in a microcentrifuge 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 and mix well.

6. Transfer the supernatant from Step 5 to a **Zymo-Spin™ IC-Z Column**[4] in a Collection Tube and centrifuge. Discard the flow-through.

---

1 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.
2 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.
3 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 C.
4 To process samples >800 µl, columns may be reloaded.

7. Add 400 µl of **ZymoBIOMICS™ DNA Wash Buffer 1** to the column in a new Collection Tube and centrifuge. Discard the flow-through.

8. Add 700 µl of **ZymoBIOMICS™ DNA Wash Buffer 2** to the column and centrifuge. Discard the flow-through.

9. Add 200 µl of **ZymoBIOMICS™ DNA Wash Buffer 2** and centrifuge the column for 1 minute to ensure complete removal of the wash buffer. Then carefully, transfer the column into a clean microcentrifuge tube.

10. Add 20 µl[1] of **ZymoBIOMICS™ DNase/RNase-Free Water** directly to the column matrix and incubate for 5 minutes. Centrifuge at full speed for 1 minute to elute the DNA.

    The DNA is now suitable for PCR and other downstream applications.

1 The final elution volume of 20 µl is recommended. Any volume from 6-100 µl can be used.

Appendices

Appendix A: Samples Collected with Swabs

Place swab directly into a clean microcentrifuge tube 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. Using a sterile method, cut the swab handle at the height of the microcentrifuge tube and leave the swab head inside the tube during (II) Host DNA Depletion, Steps 2 and 3. Carefully remove the swab from the tube after centrifugation and proceed with step 4.

Appendix B: Protocols for Other Sample Types

Liquids with Larger Volumes:

1. For liquid samples with volumes >200 µl, add 5 volumes of **Host Depletion Solution** to the sample (*Ex.* Add 5 ml of solution to 1 ml of sample).

2. Rotate sample for 15 minutes using end-over-end rotation at room temperature (20-30ºC).

3. Centrifuge the tube at 7,000 *x g* for 10 minutes, or until cells are pelleted.

4. Proceed to Step 4 of (II) Host DNA Depletion.

Whole Blood (up to 10 mL):

1. Add 3 volumes of **RBC Lysis Buffer** (#R1022-2-100, sold separately) to blood samples in a clean tube (*Ex.* Add 9 ml of buffer to 3 ml of blood in a 15 ml conical tube).

2. Mix by inverting and incubate for 5 minutes at room temperature (20-30ºC).

3. Centrifuge at 2,000 *x g* for 10 minutes to pellet cells. If available, centrifugation at 8,000 – 10,000 *x g* for 10 minutes is preferred.

4. Without disturbing the pellet, carefully remove and discard the supernatant.

5. Resuspend the pellet in 200 µl of PBS (user supplied) and proceed to Step 1 of (II) Host DNA Depletion.

Sputum:

1. Add dithiothreitol (DTT, user supplied) to sample to a final concentration of 5 mM (*Ex.* Add 10 µl of 100 mM DTT to 190 µl of sputum sample) and mix well.

2. Incubate the sample at room temperature (20-30ºC) for 15 minutes.

3. Proceed to Step 1 of (II) Host DNA Depletion.

Solid Tissues:

1. Add ≤125 mg of tissue to a **ZR BashingBead™ Lysis Tube (2.0 mm)** (#S6003-50, sold separately). Add 750 µl of **DNA Elution Buffer** (#D3004-4, sold separately) or an isotonic buffer (*e.g.* PBS) to the tube.

2. Secure in a bead beater fitted with a 2 ml tube holder assembly and process at maximum speed for 1 minute.

Note: Bead beating with 2.0 mm beads will mostly lyse tissue and host cells while preserving microbial cells. Processing times may be as little as 1 minute when using high-speed cell disrupters (FastPrep® -24).

3. Centrifuge the ZR BashingBead™ Lysis Tube (2.0 mm) in a microcentrifuge at 16,000 x *g* for 2 minutes.

4. Without disturbing the pellet, carefully remove and discard the supernatant.

5. Add 1 ml of **Host Depletion Solution** to the ZR BashingBead™ Lysis Tube (2.0 mm) and resuspend the pellet. Mix well. Transfer the mixture to a clean microcentrifuge tube.

6. Proceed to Step 2 of (II) Host DNA Depletion.

Appendix C: Microbial Composition of the ZymoBIOMICS™ Microbial Community Standard

The **ZymoBIOMICS™ Microbial Community Standard** (#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).

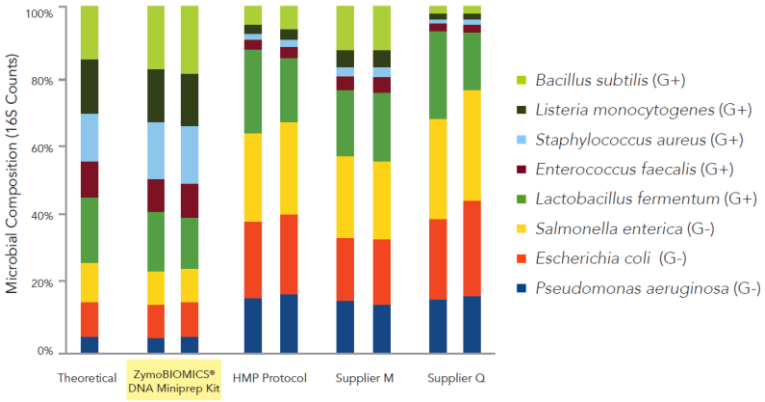


Figure 4. Benchmarking DNA extraction processes with ZymoBIOMICS™ Microbial Community Standard. DNA was extracted from ZymoBIOMICS™ Microbial Community Standard using the four different DNA extraction methods (ZymoBIOMICS™ DNA Miniprep Kit, Human Microbiome Project fecal DNA extraction protocol, a DNA 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

Appendix D: Optimized Lysis Protocols for Bead-Beating

The following conditions with different mechanical lysis machines were validated with minimum bias using the **ZymoBIOMICS™ Microbial Community Standard** (D6300).

1 Vortex Genie® with 2 ml BashingBead™ Tubes

Recommended for ease of use and accessibility

Use Microtube Adaptor (Scientific Industries, Inc. Cat. No. S5001-7)

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

2 Bertin Precellys® Evolution with 2 ml BashingBead™ Tubes

Recommended for ease of use and ultra-high speed

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

3 MP Fastprep®-24 with 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

4 Omni Bead Ruptor® Elite with 2 ml BashingBead™ Tubes

1. 1 minute on at 6 m/s
2. 5 minutes rest
3. Repeat cycle 3 times for a total of 3 minutes of bead beating

5 Biospec Mini-BeadBeater-16 with 2 ml BashingBead™ Tubes

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

6 Biospec Mini-BeadBeater-96 with 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

7 Biospec Mini-BeadBeater-96 with 96 well BashingBead™ Lysis Rack

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

X TissueLyser II

No tested conditions yielded accurate profiles. This device is not validated by Zymo Research for microbiome research.

X TissueLyser LT

No tested conditions yielded accurate profiles. This device is not validated by Zymo Research for microbiome research.

X Retsch Mixer Mill MM 400

No tested conditions yielded accurate profiles. This device is not validated by Zymo Research for microbiome research.

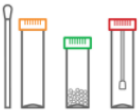
Ordering Information

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

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

Explore Other Microbiome Products

✓ To collect and transport samples at ambient temperatures:



DNA/RNA Shield™ and Collection Devices	
1X Reagent #R1100	For sample lysis and stabilization of DNA/RNA
2X Concentrate #R1200	Reagent concentrate (2X) for use with liquids at 1:1 ratio
Fecal Collection Tube #R1101	15 mL container (prefilled with 9 mL DNA/RNA Shield™). Direct collection of up to 1g or 1 mL stool
Collection Tube w/ Swab #R1106	12 x 80 mm screwcap container filled with 1 mL DNA/RNA Shield™ and sterile swab for specimen collection

✓ Unbiased and inhibitor-free DNA and RNA extraction (high-throughput and automatable) for microbial profiling:

ZymoBIOMICS™ DNA and RNA Kits	
DNA Miniprep #D4300	Up to 25 µg DNA
DNA Microprep #D4301	Up to 5 µg DNA
MagBead DNA #D4302	Automatable (Tecan, Hamilton, Kingfisher, etc.)
96-Well DNA #D4309	Spin-plate
DNA/RNA Miniprep Kit #R2002	Up to 100 µg DNA/RNA

✓ Streamlined workflows with comprehensive bioinformatics analysis and publication-ready plots and figures:

ZymoBIOMICS™ Services	
Targeted Sequencing Service 16S #Q2001	With DNA Extraction
Targeted Sequencing Service 16S #Q2012	Without DNA Extraction
Targeted Sequencing Service ITS #Q2003	With DNA Extraction
Targeted Sequencing Service ITS #Q2003	Without DNA Extraction

Troubleshooting Guide

Problem	Possible Causes and Suggested Solutions
Background Contamination	Workspace Contamination: - Clean workspace, microcentrifuge, and pipettes with 10% bleach routinely to avoid contamination. - Use of kit in exposed environment without proper filtration can lead to back ground contamination. Check pipettes, pipette tips, microcentrifuge tubes, workspace, etc. for contamination. Make sure all reagent tubes and bottles are properly sealed for storage. Use of these outside a clean room or hood can result in contamination.
Low Bacterial DNA Yield	Proteinase K Incubation: - Ensure that the incubation time does not exceed 30 minutes. Some bacterial species are sensitive to degradation by Proteinase K and will become lysed. The duration should be optimized by sample type. DNA/RNA Shield™ Incubation: - Ensure that the incubation time is at least 5 minutes and that DNA/RNA Shield™ is thoroughly mixed with the sample before proceeding. Suboptimal 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 (#D6300) to determine the best processing time and speed. - For easy-to-lyse bacteria, optimize bead beating duration and speed to prevent over-shearing of the bacterial DNA. For best results, test the lysis by beginning with shorter times and lower speeds. Buffer Mixing: - 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. Elution Procedure: - Ensure that the **ZymoBIOMICS™ DNase/RNase-Free Water** hydrates the column for at least 5 minutes before centrifugation. - To increase yields, heat the **ZymoBIOMICS™ DNase/RNase-Free Water** to 60ºC before use. Additionally, users can reload the eluate onto the column matrix, incubate at room temperature for 3 minutes, and centrifuge again to increase yield without further dilution.
High Host DNA Yield	Insufficient Incubation: - Ensure that the incubations during Host DNA Depletion are performed at the proper temperatures and for the full amount of time Insufficient Proteinase K Treatment: - Ensure that the incubation time is long enough to sufficiently deplete remaining host DNA. This should be optimized by sample type to determine sufficient depletion of host DNA and recovery of bacterial DNA.

For technical assistance, please contact 1-888-882-9682 or email tech@zymoresearch.com

Notes

100% satisfaction guarantee on all Zymo Research products, or your money back.

Zymo Research is committed to simplifying your research with quality products and services. If you are dissatisfied with this product for any reason, please call 1(888) 882-9682.

Integrity of kit 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 the highest performance and reliability.

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.

ZYMO RESEARCH

The **BEAUTY** *of* **SCIENCE** *is to Make Things* **SIMPLE**®

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682