ZYMO RESEARCH

Microbiomics
Made Simple™

# *Quick*-16S™ Plus NGS Library Prep Kit (V1-V2)

Fastest, normalization-free 16S library prep

## Highlights

- The most streamlined NGS kit with only 30 minutes of hands-on time for 96 samples.
- 100% automation ready with only a single PCR step and without the need for normalization.
- Real-time PCR enables absolute microbial quantification.

Catalog Numbers:
D6434-PS1

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

| *Quick*-16S™ Plus NGS Library Prep Kit (V1-V2) | D6434 (96 rxns.) | Storage Temp. |
|---|---|---|
| **V1-V2 Premix Plate**[1] | 13 µl each well | -20°C |
| **ZymoBIOMICS™ Microbial Community DNA Standard (50 ng)** | 10 µl | -20°C |
| **ZymoBIOMICS™ 16S/ITS qPCR Standard** | 10 µl | -20°C |
| **Select-a-Size MagBead Concentrate**[2] | 30 µl | 4-8°C |
| **Select-a-Size™ MagBead Buffer**[2] | 1 ml | 4-8°C |
| **DNA Wash Buffer** | 6 ml | Room Temp. |
| **ZymoBIOMICS™ DNase/RNase Free Water** | 1 ml | Room Temp. |
| **PCR Inactivation Solution** | 100 µl | Room Temp. |
| **Magnetic Rod** | 4 | - |
| **Instruction Manual** | 1 pc | - |

1 Protect the plate from light.
2 The Select-a-Size™ MagBead Concentrate and Buffer are shipped at room temperature but should be stored at 4-8°C upon receipt.

# Specifications

- **Sample Input –** Purified microbial DNA (≤100 ng), free of PCR inhibitors[1,2].
- **16S V1-V2 Primer Sequences (adapters not included) –** 27f (AGRGTTYGATYMTGGCTCAG, 20 bp) and 341r (CTGCWGCCHCCCGTAGG, 17 bp)
- **Index Primers –** Unique Dual Index (barcodes) to uniquely label samples.
- **Barcode Sequences –** 10 bp barcodes are available for download by visiting the Documentation section of the D6434 Product Page at www.zymoresearch.com.
- **Amplicon Size –** The final amplicon size after 1-Step PCR (targeted amplification and barcode addition) is ~492 bp.
- **Sequencing Platform –** Illumina MiSeq® or NextSeq 1000/2000® without the need to add custom sequencing primers. We recommend the MiSeq® Reagent Kit v3 (600-cycle) or NextSeq 1000/2000® Reagent Kit P1/P2 (600-cycle). For assistance with sample sheet setup, see Appendix F.
- **Equipment Needed** (user provided) **–** Microcentrifuge, plate spinner (centrifuge), 96-well real-time quantitative PCR system (SYBR Green compatible, recommended), or standard PCR system, and 96-well real-time PCR plates.
- **V1-V2 Premix Plate –** Each position on the premix plate contains Equalase™ qPCR Premix, water, and uniquely indexed (UDI) 24f and 341r primers mixed at their proper ratios for a 10 µl final reaction volume.

1 DNA that contains potent PCR inhibitors such as polyphenolics, humic/fulvic acids, tannins, melanin, etc. can be quickly cleaned using the OneStep™ PCR Inhibitor Removal Kit. See Appendix D for additional information.
2 The 1-Step PCR reaction can accommodate DNA inputs of up to 100 ng but reducing inputs to ≤10 ng is recommended for robustness against potential PCR inhibition.

# Product Description

16S rRNA gene sequencing is a routine technique for microbiome composition profiling. Compared to shotgun metagenomics sequencing, 16S rRNA gene sequencing is more cost-effective and more robust; it generally requires less input DNA and is less impacted by the presence of non-microbial DNA. However, 16S rRNA gene sequencing has its own challenges. One major challenge is the formation of PCR chimeric sequences, which are artificial sequences resulting from the recombination of two or more PCR templates. Moreover, common 16S library preparation protocols have not been optimized to be cost-effective for large-scale applications.

The ***Quick*-16S™ Plus NGS Library Prep Kit (V1-V2)** is the fastest and simplest library prep method for high-throughput 16S rRNA sequencing. Distinguishing features of the kit are described below.

**Fastest 16S Workflow.** The *Quick*-16S™ Plus NGS Library Prep Kit (V1-V2) utilizes a single qPCR/PCR for combined targeted amplification and barcode addition using specially designed primers. After pooling by equal volume, a single clean-up of the final library is performed, rather than multi-well magnetic bead clean-ups. Additional library quantification analysis such as TapeStation® analysis or gel electrophoresis is not necessary. With this workflow, the hands-on time of 16S library preparation is reduced to only 30 minutes (Figure 1).

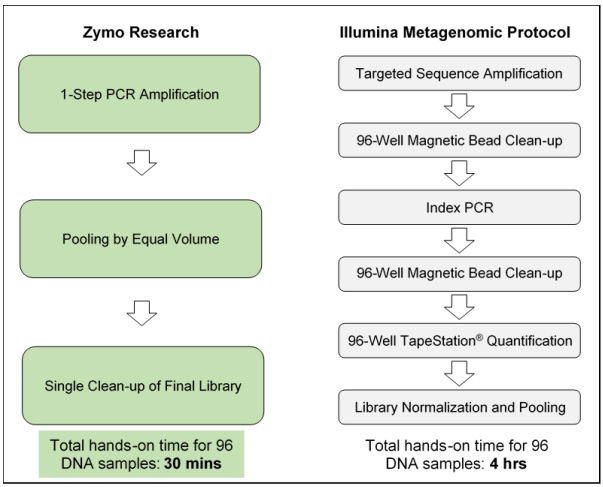



**Figure 1. *Quick*-16S™ Plus NGS Library Prep Kit (V1-V2) workflow versus the Illumina Metagenomic Protocol**. Total hands-on time calculations are based on the preparation of 96 DNA samples.

**Normalization Free.** There is no need to control sample input or PCR cycles. The workflow auto-normalizes and produces similar amounts of sequencing reads across all samples regardless of different DNA inputs (Figure 2). Just pool by equal volumes!

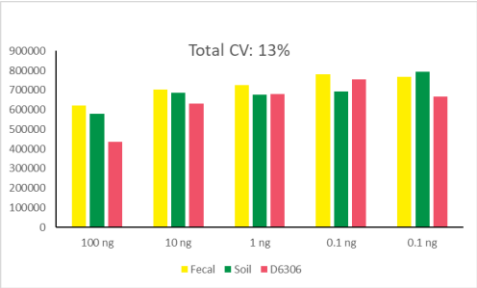



**Figure 2. The *Quick*-16S™ Plus NGS Library Prep Kit (V1-V2) results in similar amounts of reads across different input amounts without normalization.** 0.01-100 ng D6306, fecal, and soil DNA were used as inputs. Libraries were pooled by equal volumes (2 µl each) without further normalization and sequenced using the NextSeq™1000/2000 P1 Reagents (600 cycles). The CV (coefficient of variation) is the ratio of the standard deviation to the mean with lower values corresponding to less dispersion around the mean.

**High Quality NGS Library.** The workflow has been optimized to minimize amplification bias (Figure 3).

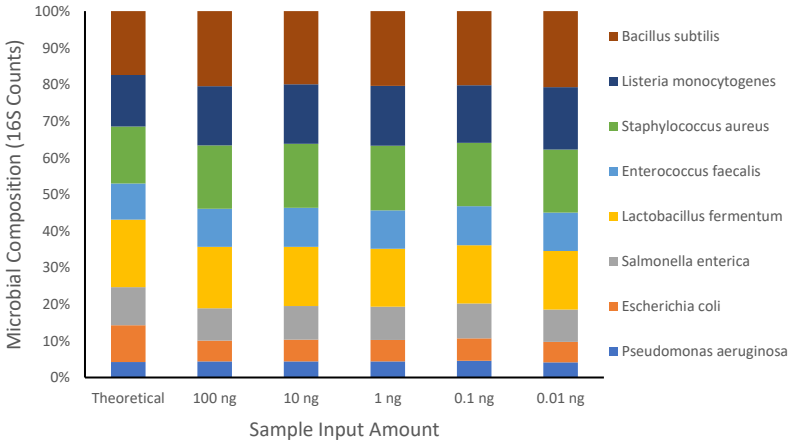



**Figure 3. Benchmarked performance with ZymoBIOMICS™ Microbial Community DNA Standard.** Bacterial composition profiles are accurate with inputs from 100 ng down to 0.01 ng.

# Protocol

## Before Starting

- ✓ **Sample Quantity Requirement.** To ensure color balance in index sequencing, a minimum of 9 samples per run is recommended.
- ✓ **Input DNA Guidelines.** All DNA samples should be free of PCR inhibitors[1]. The 1-Step PCR reaction can accommodate DNA inputs of up to 100 ng but reducing inputs to ≤10 ng is recommended for robustness against potential PCR inhibition.

## Section 1: 1-Step PCR

1. Pierce the foil and transfer 8 µl of premix from the V1-V2 Premix Plate onto a new PCR plate.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | S1 | S9 | S17 | S25 | S33 | S41 | S49 | S57 | S65 | S73 | S81 | S89* |
| B | S2 | S10 | S18 | S26 | S34 | S42 | S50 | S58 | S66 | S74 | S82 | S90* |
| C | S3 | S11 | S19 | S27 | S35 | S43 | S51 | S59 | S67 | S75 | S83 | S91* |
| D | S4 | S12 | S20 | S28 | S36 | S44 | S52 | S60 | S68 | S76 | S84 | S92* |
| E | S5 | S13 | S21 | S29 | S37 | S45 | S53 | S61 | S69 | S77 | S85 | S93* |
| F | S6 | S14 | S22 | S30 | S38 | S46 | S54 | S62 | S70 | S78 | S86 | S94* |
| G | S7 | S15 | S23 | S31 | S39 | S47 | S55 | S63 | S71 | S79 | S87 | POS** |
| H | S8 | S16 | S24 | S32 | S40 | S48 | S56 | S64 | S72 | S80 | S88 | NEG*** |

* S89-94 should be reserved for qPCR standards if absolute quantification is desired.
** POS: The **ZymoBIOMICS™ Microbial Community DNA Standard**[2] (included in kit) as a positive control.
*** NEG: A no template control as a negative control.

2. (Optional): If absolute quantification by real-time PCR is desired, add 2 µl of the serially diluted qPCR standard to the 6 wells highlighted above; S89-S94. Refer to Appendix A for more details.

*(Continued on next page.)*

1 DNA that contains potent PCR inhibitors such as polyphenolics, humic/fulvic acids, tannins, melanin, etc. can be quickly cleaned using the OneStep™ PCR Inhibitor Removal Kit. See Appendix D for additional information.
2 The composition of the microbial standard can be found in Appendix E.

3. Add 2 µl of your DNA samples to individual wells. Include a positive and a negative control on the plate.
4. Apply an adhesive PCR plate seal. Mix the plate on a plate shaker and centrifuge in a plate spinner[1].
5. Place plate in a real-time thermocycler[2] and run the program shown below:

| Temperature | Time | |
|---|---|---|
| 95°C | 10 min | Lid Temp: 105°C<br>Dye: SYBR Green |
| 95°C | 30 sec | 42 cycles |
| 55°C | 30 sec | |
| 72°C | 3 min | |
| Plate read | - | |
| 4°C | Hold | |

6. Monitor and QC the library preparation when running the reaction on a real-time thermocycler[3].
   a. For example, a sample that is expected to amplify and shows little or no amplification may indicate an error in the reaction setup (See the Troubleshooting Guide).
   b. The negative control should not amplify before 35 cycles[4]. Earlier amplification of negative control may indicate process contaminations.
   c. An example of qPCR amplification with controls is shown in Figure 5 below.

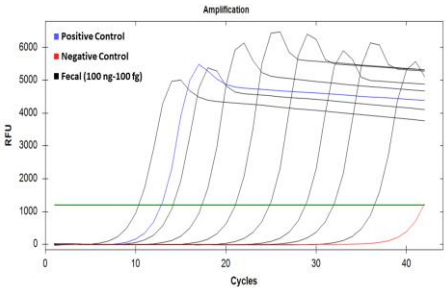



**Figure 5. qPCR Amplification Example with Positive and Negative Controls.** Serial dilutions of fecal DNA (black) from 100 ng to 100 fg were amplified on a Bio-Rad CFX96™ Real-Time PCR Detection System. The positive (blue) amplified at 12.84 and negative (red) amplified at 41.92. Baseline threshold was set at 1200 RFU.

7. Once the samples have cooled to 4°C, stop the program. Centrifuge plate in a plate spinner to collect condensation in wells and place plate on ice. Proceed to Section 2, or store plate at ≤-20°C for later use.

1 PCR reactions can be pipette mixed if a plate shaker is not available.
2 A real-time thermocycler is recommended as it enables QC of the library prep of all wells and absolute quantification as shown in Appendix A. A non-quantitative system can be used if absolute quantification is not needed.
3 If real-time PCR was not used, after amplification perform PCR cleanup for a few samples plus positive control. Analyze on a TapeStation® to confirm correct amplicon size (~492 bp).
4 The PCR program runs for 42 cycles, so it is normal to see some amplification from the negative control. The negative control should be sequenced together with other samples. If appropriate for your project, the taxa from the negative control can be subtracted from the analysis.

## Section 2: Pooling by Equal Volume

Pool equal volumes (5 µl[1]) of PCR product from each well of the plate from Section 1 into a new microcentrifuge tube2. Skip wells S89-S94 if they are used for qPCR standards. Add ~10 µl of **PCR Inactivation Solution** per 100 µl of library pool and mix well. Proceed to **Section 3**.

## Section 3: Final Library Clean-up

1. Equilibrate the **Select-a-Size MagBead Buffer** to room temperature (15-30°C). Add 30 µl of **Select-a-Size MagBead Concentrate** to the 1 ml Select-a-Size MagBead Buffer. Resuspend the magnetic particles by vigorously shaking until homogenous.
2. Add **Select-a-Size™ MagBeads** to the pooled library from Section 2 at a ratio of 0.8x volume. For example, add 400 µl of Select-a-Size™ MagBeads to 500 µl of the pooled library and PCR Inactivation Solution mixture.
3. Mix thoroughly by pipetting or vortexing until homogenous. Incubate for 5 minutes at room temperature.
4. Place the sample on a magnetic rack[3] and incubate for 3-10 minutes at room temperature, or until the magnetic beads have fully separated from solution.
5. Once the beads have cleared from solution, remove and discard the supernatant[4].
6. While the beads are still on the magnetic rack, add 1 ml of **DNA Wash Buffer**. Remove and discard the supernatant. Repeat this step.
7. While the beads are still on the magnetic rack, aspirate out any residual buffer with a 10 µl pipette tip.
8. Remove tube from the magnetic rack and keep the cap open for 3 minutes at room temperature to dry the beads.

*(Continued on next page.)*

1 If pooling >96 samples, use 2 µl instead to accommodate all samples.
2 There is no need for additional normalization procedures. To obtain better normalization and adjust pooling volumes, See Appendix B.
3 Alternatively, the provided Magnetic Rod can be used.
4 Avoid aspirating any beads when removing the supernatant. To best prevent this, leave 2-5 µl of liquid behind.

9. Add 10-100 µl[1] of **ZymoBIOMICS™ DNase/RNase Free Water** to the beads and pipette mix thoroughly. Incubate at room temperature for 2 minutes.

10. Place the sample on a magnetic rack and incubate for 1 minute at RT, or until the magnetic beads have fully separated from eluate.

11. Transfer supernatant to a clean microcentrifuge tube. Proceed to **Section 4**.

## Section 4: Library Quantification

Use a fluorescence-based method (Qubit® dsDNA HS Assay Kit recommended) to quantify the final library. Using a final amplicon size of 492 bp, convert ng/µl to nM using the equation below.

$$\frac{concentration\ in\ ng/ul}{660\ g/mol\ x\ average\ library\ size\ in\ bp}\ x\ 10^6\ =\ concentration\ in\ nM$$

For example: 20 ng/µl DNA of the final library is equivalent to 61.6 nM.

If preferred, a qPCR-based method for quantification such as the KAPA® Library Quantification kit may be used.

***DNA Fragment Analysis (Not Required)***

If a fragment analyzer (e.g. TapeStation®) is used to analyze the final library, there may be a lack of a tight band at ~500 bp. Because of the library prep design, some library products have run through additional PCR cycles and might not anneal well. They are perfectly fine for sequencing, which already denatures double-stranded DNA into single strands.

---

**This is your final 16S library.**

The ultra-pure pooled library DNA is now ready for use or storage at ≤-20ºC. Refer to platform-specific guidelines for preparation for sequencing. No custom sequencing primers are needed.

---

**Illumina Loading Parameters:**

For the MiSeq® Reagent Kit v3 (600-cycle), a final library loading concentration of 10-12pM[2] with 15% PhiX spike-in is recommended. For the NextSeq® Reagent Kit P1/P2 (600-cycle), a final library loading concentration of 750 pM using onboard denature and dilute with 40% PhiX spike-in is recommended.

1 If pooling fewer than 10 samples, use 10 µl for elution.
2 Optimal loading concentration may vary by instrument. Adjust final library loading concentrations as needed to reach a target cluster density of 700 K/mm$^2$.

# Appendices

## Appendix A: Absolute Quantification of Total 16S Copy Number by Real-Time PCR

Absolute quantification is determined with a real-time PCR standard curve using serially diluted amounts of the ZymoBIOMICS™ 16S/ITS qPCR Standard.

1. Dilute the ZymoBIOMICS™ 16S/ITS qPCR Standard ($7.5x10^6$ copies/µl) to $7.5x10^6$ copies/µl, $7.5x10^4$ copies/µl and $7.5x10^2$ copies/µl.

2. Add 2 µl of the standard and its dilutions to six wells (each in duplicate in positions S89-S94; see plate layout on Page 6) on the same plate that contains unknown samples for library prep.

3. After 1-Step PCR, plot the concentration (16S copies/µl) of the standards against the Ct values to produce a standard curve. See Figure 6 and 7 for an example.

4. Calculate the total 16S copy number of unknown samples based on their Ct value with the standard curve accordingly[1].

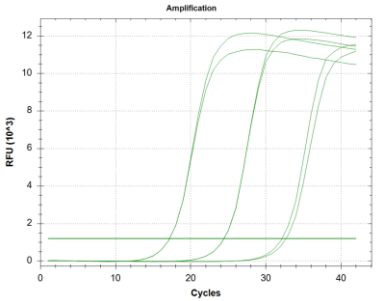



**Figure 6. qPCR of Diluted Amounts of 16S/ITS Standard.** The standard was amplified on a Bio-Rad CFX96™ Real-Time PCR Detection System. Baseline threshold was set at 1200 RFU.

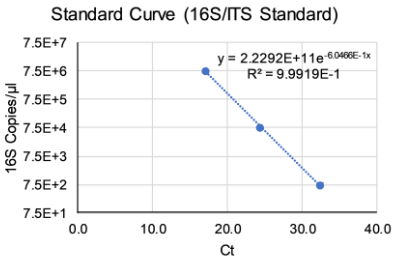



**Figure 7. Absolute Quantification using a Standard Curve.** Ct can be correlated to the total 16S copies based on the regression.

1 Excel template for the Absolute Quantification of Total 16S Copy Number is available for download by visiting the Documentation section of the D6434 Product Page at www.zymoresearch.com.

## Appendix B: Improving Read Distribution by Fine-Tuning Pooling Volumes based on qPCR

Typically, pooling samples by equal volumes will result in sufficient normalization and similar number of reads per sample. To obtain even better normalization, the pooling volumes can be adjusted based on Ct values obtained.

First, adjust the fluorescence threshold/cutoff to a certain value so that the positive control[1] has a Ct of 15. Use the following equation to calculate the pooling volume of each well based on its Ct: Volume (µl) = $0.000598x^2 - 0.0637x + 3.178$; x = Ct. For example, the pooling volumes for Ct values of 15, 20, and 30 are 2.36 µl, 2.14 µl, and 1.81 µl respectively. This strategy can further reduce the CV (coefficient of variation) of reads assigned to samples by 3-6%.

## Appendix C: Index Primer Sets

To accommodate sequencing projects of various sizes, Zymo Research offers primer sets that can uniquely barcode more than 96 samples. The barcodes of each index primer are distinct from one another by at least 5 bp to boost the accuracy of demultiplexing. For projects that require indexes for >96 samples, please contact Zymo Research Corporation, and we can assist with designing additional barcoding primers.

## Appendix D: Removal of PCR Inhibitors from Starting DNA

The input DNA samples for the *Quick*-16S™ Plus NGS Library Prep Kit (V1-V2) must be free of PCR inhibitors such as polyphenolics, humic/fulvic acids, tannins, melanin, etc. To further remove PCR inhibitors from purified DNA samples, Zymo Research recommends performing a one-step cleanup with the OneStep™ PCR Inhibitor Removal Kit. Additional information can be found by visiting the D6030 Product Page at www.zymoresearch.com.

1 The positive control is the sample that uses 2 µl of the ZymoBIOMICS™ Microbial Community DNA Standard.

## Appendix E: Composition of ZymoBIOMICS™ Microbial Community DNA Standard (50 ng)

The **ZymoBIOMICS™ Microbial Community DNA Standard (50 ng)** is a mixture of genomic DNA extracted from pure cultures of eight bacterial and two fungal strains. Genomic DNA from each culture is quantified before mixing. The ZymoBIOMICS™ Microbial Community DNA Standard allows for assessment of bias from library preparation, sequencing, and bioinformatics analysis. More information about the standard can be found below.

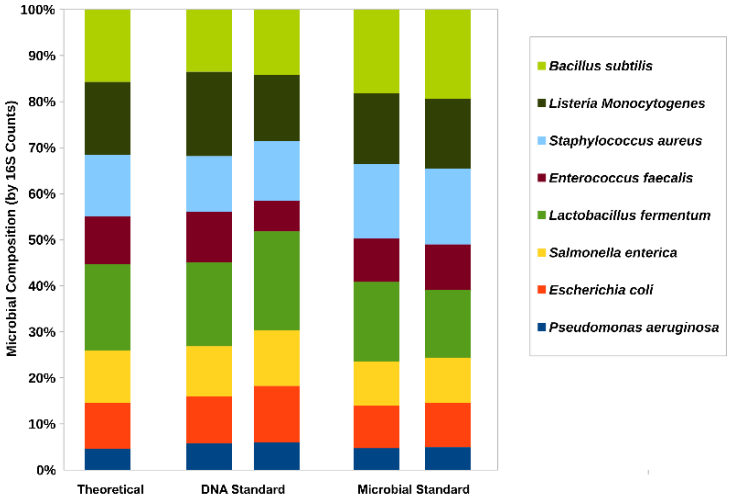



**Figure 8. Accurate composition for reliable use to evaluate 16S rRNA sequencing.** Characterization of the microbial composition of the two ZymoBIOMICS™ standards by 16S rRNA gene targeted sequencing. The measured composition of the two standards agrees with the theoretical/designed composition. "DNA Standard" represents ZymoBIOMICS™ Microbial Community DNA Standard and "Microbial Standard" represents ZymoBIOMICS™ Microbial Community Standard. 16S composition by 16S rRNA gene targeted sequencing was calculated based on counting the amount of 16S raw reads mapped to each genome.

## Appendix F: Illumina MiSeq® Sample Sheet Setup

A template for the Illumina MiSeq® sample sheet is available for download by visiting the Documentation section of the Product Page at www.zymoresearch.com. Fill in the project and sample information in the highlighted fields, then save the file in comma-separated values (CSV) format for use with the Illumina MiSeq®.

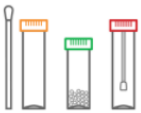
# Ordering Information

| Product Description | Catalog No. | Size / Format |
|---|---|---|
| ***Quick*-16S™ Plus NGS Library Prep Kit (V1-V2)** | D6434-PS1 | 96 rxns. / Primer Set 1 |

| Individual Kit Components | Catalog No. | Amount |
|---|---|---|
| **ZymoBIOMICS™ DNase/RNase Free Water** | D4302-5-10 | 10 ml |
| **ZymoBIOMICS™ Microbial Community DNA Standard (200 ng)** | D6305 | 200 ng |
| **ZymoBIOMICS™ Microbial Community DNA Standard (2000 ng)** | D6306 | 2000 ng |

# Explore Other Microbiome Products

✓ To collect and transport samples at ambient temperatures:

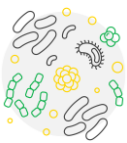


| DNA/RNA Shield™ and Collection Devices | |
|---|---|
| 1X Reagent #R1100 | For sample lysis and stabilization of DNA/RNA |
| 2X Concentrate #R1200 | Reagent concentrate (2X) for use with liquids at 1:1 ratio |
| Fecal Collection Tube #R1101 | 15 mL container (prefilled with 9 mL DNA/RNA Shield™). Direct 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 |

✓ Microbial standards and references for profiling quality control, benchmarking, positive controls, and to assess performance of entire microbiomic/metagenomic workflows:



| ZymoBIOMICS™ Standards and Reference Materials | |
|---|---|
| Microbial Community Standard #D6300 | Contains 8 bacteria and 2 yeasts for QC and method optimization |
| Microbial Community DNA Standard #D6305 | Contains 8 bacteria and 2 yeasts DNA for bioinformatics optimization |
| Gut Microbiome Standard #D6331 | Contains 21 different human gut strains for method benchmarking |
| Fecal Reference with TruMatrix™ Technology #D6323 | Contains real human fecal material for benchmarking and improved data reproducibility |

# Troubleshooting Guide

| Problem | Possible Causes and Suggested Solutions |
|---|---|
| **Background Contamination** | Workspace contamination:<br>- Clean workspace, microcentrifuge, and pipettes with 10% bleach routinely to avoid contamination.<br>- Use of kit in exposed environment without proper filtration can lead to background contamination. Check pipettes, pipette tips, microcentrifuge tubes, workspace, etc. for contamination<br>- 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. |
| **Loss of Volume during PCR** | Adhesive seal:<br>- A loosened adhesive seal on the PCR plate can lead to sample evaporation. Ensure that the plate seal is secure on every well during targeted sequence amplification.<br>Lid pressure:<br>- Inconsistent lid pressure. Ensure that the lid pressure on the real-time quantitative PCR instrument is consistent over the PCR plate according to the manufacturer's recommendation. |
| **Unexpected or No Amplification of DNA Sample During PCR Program in Section 1** | Sample with high microbial DNA concentration:<br>- Reaction setup error. A sample that is expected to amplify but shows little or no amplification during the PCR program in **Section 1** may indicate an error in the reaction setup. Use a new aliquot of the sample and repeat **Section 1**.<br>- Sample may contain high levels of PCR inhibitors. See Appendix D on how to remove these and repeat **Section 1**. Additionally, samples may be diluted to lower concentration (<10 ng/µl) to see if that improves amplification efficiency.<br>Sample with low microbial DNA concentration:<br>- Check negative control. A sample with little microbial DNA may not amplify before the negative control. Either use more concentrated DNA or use more DNA volume during reaction setup.<br>Abnormal qPCR curves:<br>- Proceed as normal. Abnormal qPCR amplification curves may occur, and this is normal performance. This is usually a slight dip in RFU (forming a small "hump"). |
| **Diminished Amplicon Bands in Library Analysis** | No single amplicon peak and/or high background:<br>- Proceed as normal. There may be a lack of a single band and/or high background if using TapeStation® or similar methods to determine amplicon size. This is normal and is part of the library prep design. Do not use this sizing and quantification data. To properly quantify library, use a fluorescence-based method and calculation in **Section 4**. |

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

# Notes

# 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