ZYMO RESEARCH

RNA Purification Made Simple™

ZymoBIOMICS™ DNA/RNA Miniprep Kit

Microbiome DNA and RNA from any sample

Highlights

- **ZymoBIOMICS™** innovative lysis system enables efficient and unbiased lysis of microbes including gram positive/negative bacteria, fungi, protozoans, and viruses from any sample including feces, soil, plant, water, biofilms, swabs, saliva, body fluids, etc.
- Rapid and robust, spin-column purification of high-quality DNA/RNA (including small/microRNAs) that is inhibitor-free and ready for RT/qPCR and microbiome measurements using Next-Gen sequencing.
- High-sensitivity and increased detection limit of very low abundance organisms

Catalog Numbers:
R2002

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

ZymoBIOMICS™ DNA/RNA Miniprep Kit	R2002 (50 prep)
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	50
DNA/RNA Shield™	50 ml
DNA/RNA Lysis Buffer	50 ml
DNA/RNA Prep Buffer	50 ml
DNA/RNA Wash Buffer[1] (concentrate)	24 ml (x2)
ZymoBIOMICS™ DNase/RNase-Free Water	30 ml
ZymoBIOMICS™ HRC Prep Solution	30 ml (x3)
DNase I[2] (lyophilized)	250 U
DNA Digestion Buffer	4 ml
Zymo-Spin™ III-HRC Filters	100
Spin-Away™ Filters	50
Zymo-Spin™ IIICG Columns	50
Collection Tubes	300
Instruction Manual	1

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature.
Before use:
1 Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA/RNA Wash Buffer** concentrate.
2 Reconstitute lyophilized **DNase I** with **ZymoBIOMICS™ DNase/RNase-Free Water**, mix by gentle inversion and store frozen aliquots:
#E1009-A (250 U), add 275 µl **water**

Specifications

- **Sample Sources –** Bacterial, fungal, protozoan, algae, viral, mitochondrial, and host DNA and RNA is efficiently isolated from ≤ 250 mg of soil, mammalian feces and plant/seed, ≤ 50-100 mg (wet weight) fungal bacterial cells[1], biofilms, water, and swabs.

- **Sample Homogenization – ZymoBIOMICS™** innovative lysis system ensures complete lysis of the microbial cell walls and accurate microbial analysis, free of bias.

- **Sample Preservation – DNA/RNA Shield™** lyses cells, inactivates nucleases and infectious agents, and is ideal for sample storage and transport at ambient temperatures.

- **Size –** DNA and total RNA including small/microRNAs (≥ 17 nt).

- **Purity –** A_{260}/A_{280} & A_{260}/A_{230} > 1.8. DNA and RNA is ready for Next-Gen Sequencing, RT/qPCR, etc.

- **Binding Capacity –** 100 µg DNA/RNA (**Zymo-Spin™ IIICG Column**).

- **Elution Volume –** ≥ 50 µl **ZymoBIOMICS™ DNase/RNase-Free Water**.

- **Equipment Needed** (user provided) **–** Microcentrifuge, vortex, cell-disruptor (recommended).

- **Recommended Materials** (available separately) **–**

  DNA/RNA Shield™ collection devices:
  fecal collection tube; R1101
  collection tube; R1102
  lysis tube (microbe); R1103
  lysis tube (microbe) w/ swab; R1104
  lysis tube (tissue); R1105
  collection tube (1 ml fill) w/ swab; R1106, R1107
  collection tube (2 ml fill) w/ swab; R1108, R1109

---

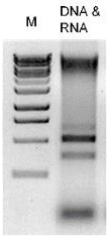
1 This equates to approximately 10^9 bacterial cells, 10^8 yeast cells, and 10^7 mammalian cells.

Product Description

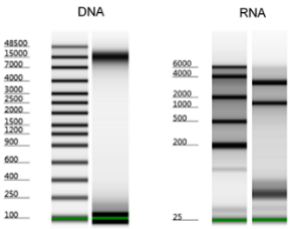
The **ZymoBIOMICS™ DNA/RNA Miniprep Kit** is designed for purifying DNA and RNA from a wide array of sample inputs (e.g. feces, soil, plant, water, and biofilms) that is ready for microbiome or metagenome analyses. The **ZymoBIOMICS™** innovative lysis system eliminates bias associated with unequal lysis efficiencies of different organisms (e.g. gram negative/positive bacteria, fungus, protozoans, and algae). The provided **DNA/RNA Shield™** preserves nucleic acids at ambient temperatures, providing an unbiased molecular snapshot of the sample.

The procedure uses **Zymo-Spin™** column technology that results in high-quality DNA and total RNA (including small/microRNAs 17-200 nt) that is free of PCR inhibitors (e.g. polyphenols, humic acids and fulvic acids) and is ready for RT-PCR, arrays, sequencing, etc.

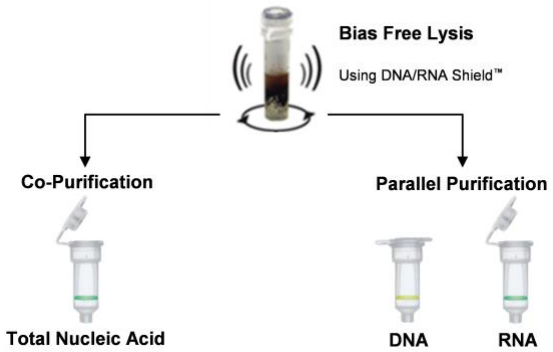
Efficient DNA and RNA Recovery



Human stool total nucleic acid (DNA & RNA) isolated with the **ZymoBIOMICS™ DNA/RNA Miniprep Kit** is high quality. Elutions were analyzed in a 1% TAE/agarose/EtBr gel. The size marker "M" is a 1 kb ladder (Zymo Research).



Human stool genomic DNA and total RNA isolated with the **ZymoBIOMICS™ DNA/RNA Miniprep Kit** is highly intact. Quality assessed by Agilent 2200 TapeStation™.



Protocol

The protocol consists of: (I) Buffer Preparation, (II) Sample Preparation, (III) Total Nucleic Acid Purification and (IV) DNA and RNA Purification

(I) Buffer Preparation

- ✓ Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA/RNA Wash Buffer** concentrate.
- ✓ Reconstitute lyophilized **DNase I** with **ZymoBIOMICS™ DNase/RNase-Free Water**, mix by gentle inversion and store frozen aliquots:
  #E1009-A (250 U), add 275 µl **water**

(II) Sample Preparation

- ✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.
- ✓ The sample input can be scaled up or down, proportionally.

1. Add 750 µl **DNA/RNA Shield™** to a sample (see table below) in a **ZR BashingBead Lysis Tube (0.1 & 0.5 mm)** and cap tightly. If a sample is already collected in **DNA/RNA Shield™**, proceed to step 2.

Soil, feces, plant, seed	≤ 250 mg
Cells in DNA/RNA Shield™ or isotonic buffer/PBS (bacterial 10^9, yeast 10^8, mammalian 10^7)	≤ 50-100 mg (wet weight)
DNA/RNA Shield™ collection devices (e.g., cat. #R1101, R1102-R1105) or **Biological liquids and swab collections** (e.g., cat. #R1100, R1106-R1109, R1150)	≤ 200 µl

2. For complete lysis of tough-to-lyse samples (microbes, tissue, etc.), perform mechanical homogenization (e.g., mortar/pestle, dounce, syringe, tissue grinder, or bead beating (recommended)).

   Secure lysis tube in a high-speed bead beater fitted with a 2 ml tube holder assembly (e.g., MP Bio FastPrep-24, Bertin Precellys, etc.) and process[1] at maximum speed for ≥ 5 minutes.

3. Centrifuge and transfer up to 200 µl of the supernatant[2] into a nuclease-free tube (not provided).

4. Add an equal volume of **DNA/RNA Lysis Buffer** to the supernatant[2] (1:1) and mix well. Then proceed to Total Nucleic Acid Purification (page 6) or DNA and RNA Purification (page 7).

1 Processing time will vary based on sample input and bead beater. For low-speed homogenizers (e.g., Disruptor Genie), process samples for ≥ 15 minutes. Optimization may be required.
2 Up to 200 µl sample can be processed per prep without reloading the column.

(III) Total Nucleic Acid Purification

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

1. Add an equal volume of ethanol (95-100%) to the sample (1:1) and mix.
2. Transfer the mixture into a **Spin-Away™ Filter**[1] (**yellow**) in a **Collection Tube** and centrifuge. Discard the flow-through.
3. Add 400 µl **DNA/RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
4. Add 400 µl **DNA/RNA Wash Buffer** to the column and centrifuge. Carefully, transfer the column into a nuclease-free tube (not provided).
5. Add 100 µl **ZymoBIOMICS™ DNase/RNase-Free Water** directly to the column matrix, incubate for 5 minutes, and then centrifuge to elute.
6. Add 2 volumes of **DNA/RNA Lysis Buffer** to the sample (2:1) and mix.
7. Add an equal volume of ethanol (95-100%) (1:1) and mix.
8. Transfer the sample into a **Zymo-Spin™ IIICG Column**[1] (**green**) in a **Collection Tube** and centrifuge. Discard the flow-through.
9. Add 400 µl **DNA/RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
10. Add 700 µl **DNA/RNA Wash Buffer** and centrifuge. Discard the flow-through.
11. Add 400 **DNA/RNA Wash Buffer** and centrifuge the column for 2 minutes to ensure complete removal of the wash buffer. Carefully transfer the column into a new nuclease-free tube (not provided).
12. Add 100 µl **ZymoBIOMICS™ DNase/RNase-Free Water** directly to the column matrix, incubate for 5 minutes. Then centrifuge to elute.

    Alternatively, for highly concentrated DNA/RNA use ≥ 50 µl elution.
13. Place a **Zymo-Spin™ III-HRC Filter** in a **Collection Tube** and add 600 µl **ZymoBIOMICS™ Prep Solution**. Centrifuge at 8,000 x *g* for 3 minutes.
14. Transfer the eluted DNA/RNA (step 12) into a prepared **Zymo-Spin™ III-HRC Filter** in a nuclease-free tube (not provided). Then centrifuge at exactly 16,000 x g for 3 minutes.

    The filtered DNA/RNA can be used immediately or stored frozen.

---

1 To process samples > 700 µl, **Zymo-Spin™** columns may be reloaded.

(IV) DNA and RNA Purification (in two separate fractions)

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

1. Transfer the sample into a **Spin-Away™ Filter**[1] (**yellow**) in a **Collection Tube** and centrifuge. **SAVE** the **flow-through** for RNA and the **column** for DNA purification!

DNA Purification
(DNA is bound to the column)

2. Transfer the **Spin-Away™ Filter** (**yellow**) into a new **Collection Tube**.

RNA Purification
(RNA is in the flow-through)

2. Add an equal volume of ethanol (95-100%) to the flow-through (1:1) and mix well. Then transfer the sample into a **Zymo-Spin™ IIICG Column**[1] (**green**) in a **Collection Tube** and centrifuge. Discard the flow-through.

   At this point, **DNase I** treatment (in-column) can be performed (page 8).

3. Add 400 µl **DNA/RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
4. Add 700 µl **DNA/RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.
5. Add 400 µl **DNA/RNA Wash Buffer** to the column and centrifuge for 2 minutes to ensure complete removal of the wash buffer. Carefully transfer the column into a nuclease-free tube (not provided).
6. Add 100 µl **ZymoBIOMICS™ DNase/RNase-Free Water** directly to the column matrix, incubate for 5 minutes, and then centrifuge to elute DNA and RNA from the respective column.

   Alternatively, for highly concentrated DNA and RNA use ≥ 50 µl elution.

7. Place **Zymo-Spin™ III-HRC Filter** in a **Collection Tube** and add 600 µl **ZymoBIOMICS™ HRC Prep Solution**. Centrifuge at 8,000 x g for 3 minutes.
8. Transfer the eluted DNA and RNA (step 6) into a prepared **Zymo-Spin™ III-HRC Filter** in a nuclease-free tube (not provided). Then centrifuge at exactly 16,000 x g for 3 minutes.

   The filtered DNA and RNA can be used immediately or stored frozen.

---

1 To process samples > 700 µl, **Zymo-Spin™** columns may be reloaded.

Appendices

Samples stabilized and stored in DNA/RNA Shield™

Recommended: **DNA/RNA Shield™** effectively lyses cells, inactivates nucleases and infectious agents and is ideal for sample storage/transport at ambient temperatures prior to nucleic acid purification.

Liquid samples: Mix an equal volume **DNA/RNA Shield™** (2X concentrate) and sample (1:1).
Solid samples: Submerge sample (not to exceed 10% (v/v or w/v) in **DNA/RNA Shield™** (1X).

Mix well/homogenize sample prior to storage. Samples in **DNA/RNA Shield™** can be stored at ambient temperature ≥ month or long term at frozen temperature.

DNase I Treatment (in-column)

1. Following RNA binding step (page 7, step 2), add 400 µl **DNA/RNA Wash Buffer** to the column, centrifuge and discard the flow-through.
2. For each sample to be treated, prepare **DNase I Reaction Mix** in a nuclease-free tube (not provided) and mix by gentle inversion. Then add 80 µl directly into column matrix and incubate at room temperature (20-30°C) for 15 minutes. Proceed with the purification protocol (page 7, step 3).

DNase I Reaction Mix	
DNase I (reconstituted; 1 U/ul)[1]	5 µl
DNA Digestion Buffer	75 µl

1 Unit definition – one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A260 units/ml of reaction mixture at 25°C.

Ordering Information

Product Description	Catalog No.	Size
ZymoBIOMICS™ DNA/RNA Miniprep Kit	R2002	50 preps.

Individual Kit Components	Catalog No.	Amount
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	S6012-50	50
DNA/RNA Shield™	R1100-50 R1100-250	50 ml 250 ml
DNA/RNA Lysis Buffer	D7001-1-50	50 ml
DNA/RNA Prep Buffer	D7010-2-25 D7010-2-50	25 ml 50 ml
DNA/RNA Wash Buffer (concentrate)	D7010-3-12 D7010-3-24	12 ml 24 ml
ZymoBIOMICS™ DNase/RNase-Free Water	D4302-5-30 D4302-5-50	30 ml 50 ml
DNase I Set (lyophilized) DNase I (250 U) & DNA Digestion Buffer (4 ml)	E1010	1 set
OneStep™ PCR Inhibitor Removal Kit	D6030	50
Spin-Away™ Filters	C1006-50-F	50
Zymo-Spin™ IIICG Columns	C1006-50-G	50
Collection Tubes	C1001-50 C1001-500	50 500
DNA/RNA Shield™ - Fecal Collection Tube	R1101	10
DNA/RNA Shield™ Collection Tube **DNA/RNA Shield™ Lysis Tube** (microbe) **DNA/RNA Shield™ Lysis Tube** (microbe) w/ swab **DNA/RNA Shield™ Lysis Tube** (tissue)	R1102 R1103 R1104 R1105	50 50 50 50
DNA/RNA Shield™ Collection Tube (1 ml fill) w/ swab	R1106 R1107	10 50
DNA/RNA Shield™ Collection Tube (2 ml fill) w/ swab	R1108 R1109	10 50

Complete Your Workflow

✓ For tough-to-lyse samples, use ZR BashingBead Lysis Tubes:

ZR BashingBead Lysis Tubes	
2.0 mm beads #S6003	For plant/animal tissue
0.1 + 0.5 mm beads #S6012	For microbes
0.1 + 2.0 mm beads #S6014	For microbes in tissue/insects

✓ For high-throughput and automatable microbiome DNA and RNA purification from any sample (DNase I Set included):

ZymoBIOMICS DNA/RNA	
MagBeads #R2135, R2136	Automatable (Tecan, Hamilton, Kingfisher, etc.)

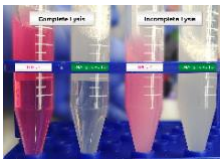
✓ For RNA clean-up (purification) from the aqueous phase (e.g., TRIzol, TRI Reagent or similar) or from any enzymatic reaction (e.g., DNase I treated RNA):

RNA Clean & Concentrator kit	
Spin-column #R1013-R1014	DNase I Set included
MagBeads #R1081, R1082	Automatable (Tecan, Hamilton, Kingfisher, etc.)

✓ For NGS:

Zymo-Seq RiboFree Total RNA Library Prep kit	
#R3000	12 preps
#R3003	96 preps

Troubleshooting Guide

Problem	Possible Causes and Suggested Solutions
Precipitation, viscous lysate	Incomplete lysis and/or high-mass input: - If precipitation occurs (upon adding ethanol to the lysate) or if the lysate is extremely viscous, increase the volume of DNA/RNA Shield and/or DNA/RNA Lysis Buffer to ensure complete lysis and homogenization until lysate is transparent (see image). 
Low purity (A_{260}/A_{230} nm, A_{260}/A_{280} nm)	Sample handling: - Ethanol and/or salt contamination. After centrifugation steps, carefully remove the column from the collection tube to prevent buffer carryover. Alternatively, blot emptied collection tube with a tissue or towel. - Make sure lysate and wash buffers have passed completely through the matrix of the column. This may require centrifuging at a higher speed and/or longer time. Incomplete lysis and/or cellular debris: - Increase the volume of DNA/RNA Shield and/or DNA/RNA Lysis Buffer to ensure complete lysis and homogenization. Be sure to centrifuge any cellular debris and then process the cleared lysate.
Low yield	Sample input: - Too much input or incomplete lysis/homogenization can cause cellular debris to clog or overload the column and result in compromised RNA recovery. Use less input material and/or increase DNA/RNA Shield and/or DNA/RNA Lysis Buffer.
DNA contamination	To remove DNA: - Perform in-column DNase I treatment or perform DNase I treatment post-purification (R1013, page 4), then clean-up the treated sample.
RNA degradation	To prevent RNA degradation: - Immediately collect and lyse fresh sample into DNA/RNA Shield to ensure nucleic acid stability. Homogenized samples in DNA/RNA Shield can be stored frozen for later processing.

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

Notes

Notes

Notes

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