ZYMO RESEARCH

RNA Purification Made Simple™

ZymoBIOMICS™ RNA Miniprep Kit

Microbiome 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 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:
R2001

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

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ZymoBIOMICS™ RNA Miniprep Kit	R2001 (50 prep)
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	50
DNA/RNA Shield™	50 ml
RNA Lysis Buffer	50 ml
RNA Prep Buffer	25 ml (x2)
RNA Wash Buffer[1] (concentrate)	24 ml
ZymoBIOMICS™ DNase/RNase-Free Water	30 ml
ZymoBIOMICS™ HRC Prep Solution	30 ml
DNase I[2] (lyophilized)	250 U
DNA Digestion Buffer	4 ml
Zymo-Spin™ III-HRC Filters	50
Zymo-Spin™ IIICG Columns	100
Collection Tubes	150
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 **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 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 –** Total RNA including small/microRNAs (≥ 17 nt).

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

- **Binding Capacity –** 100 µg 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.

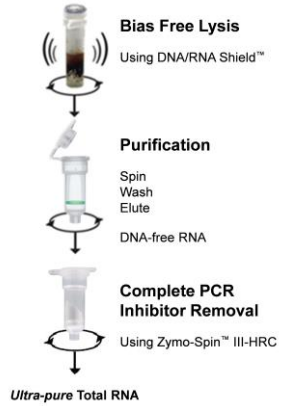
Product Description

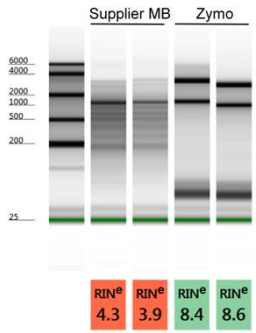
The **ZymoBIOMICS™ RNA Miniprep Kit** is designed for purifying RNA from a wide array of sample inputs (e.g. feces, soil, plant, water, and biofilms) that is ready for microbiome or metagenome analyses.

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.

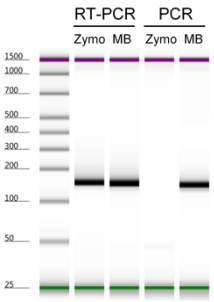
The procedure uses **Zymo-Spin™** column technology that results in high-quality total RNA (including small/microRNAs 17-200 nt) that is free of PCR inhibitors (e.g. polyphenols, humic acids and fulvic acids) and is ready for RT-PCR, arrays, sequencing, etc.

High-Quality RNA





Human stool RNA isolated with the **ZymoBIOMICS™ RNA Miniprep Kit** is higher quality (right); compared to Supplier MB procedures (left). Quality assessed by Agilent 2200 TapeStation™.



Human stool RNA was analyzed after RT-PCR and PCR amplification (~150 bp fragment shown) for both Zymo and Supplier MB procedures. Lack of a band in PCR using the **ZymoBIOMICS™ RNA Miniprep Kit** indicates DNA-free RNA. Quality assessed by Agilent 2200 TapeStation™.

Protocol

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

(I) Buffer Preparation

- ✓ Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **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™**, transfer 750 µl liquid sample into a **ZR BashingBead Lysis Tube (0.1 & 0.5 mm)** and cap tightly.

Sample Type	Maximum Input
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 swabs collected in DNA/RNA Shield™** (e.g., cat. #R1100, R1106-R1109, R1150)	750 µl

2. For complete lysis of tough-to-lyse samples (microbes, tissue, etc.), perform mechanical homogenization in a **ZR BashingBead Lysis Tube (0.1 & 0.5 mm)** by securing in a high-speed bead beater fitted with a 2 ml tube holder assembly (e.g., MP Bio FastPrep-24, Bertin Precellys, etc.). Process[1] at maximum speed for ≥ 5 minutes.
3. Centrifuge and transfer up to 400 µl of the supernatant[2] into a nuclease-free tube (not provided).
4. Add an equal volume of **RNA Lysis Buffer** to the supernatant[2] (1:1) and mix well. Then proceed to purification (page 6).

1 Processing time will vary based on sample input and bead beater. For low-speed homogenizers (e.g., Vortex Genie), process samples for ≥ 15 minutes. Optimization may be required.
2 Up to 400 µl sample input can be processed per prep.

(III) Total RNA 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 in **RNA Lysis Buffer** and mix well.

   Example: Add 800 µl ethanol to 800 µl mixture (sample mixed in **RNA Lysis Buffer**).

2. Transfer the mixture into a **Zymo-Spin™ IIICG Column**[1] (**green**) in a **Collection Tube** and centrifuge. Discard the flow-through.

3. Add 400 µl **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.

4. Add 400 µl **RNA Wash Buffer** to the column and centrifuge. Carefully, transfer the column into a nuclease-free tube (not provided).

5. Add 85 µl **ZymoBIOMICS™ DNase/RNase-Free Water** directly to the column matrix, then centrifuge to elute.

6. **DNase I**[2] treatment (recommended)
   (D1) To the eluate, add 10 µl **DNA Digestion Buffer** and 5 µl **DNase I** and mix gently by inversion.
   (D2) Incubate at room temperature (20-30°C) for 15 minutes.

7. Add 2 volumes of **RNA Lysis Buffer** to the sample (2:1) and mix.

   Example: Add 200 µl **RNA Lysis Buffer** to 100 µl mixture (**DNase I**-treated eluate).

8. Add an equal volume of ethanol (95-100%) (1:1) and mix.

   Example: Add 300 µl ethanol to 300 µl mixture (eluate in **RNA Lysis Buffer**).

9. Transfer the mixture into a new **Zymo-Spin™ IIICG Column**[1] (**green**) in a **Collection Tube** and centrifuge. Discard the flow-through.

10. Add 400 µl **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.

(continue to purification, page 7)

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1 To process sample volume > 700 µl, **Zymo-Spin™**, columns may be reloaded.
2 Prior to use, reconstitute the lyophilized **DNase I** (Buffer Preparation, page 4). * Unit definition – one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A_{260} units/ml of reaction mixture at 25°C.

11. Add 700 µl **RNA Wash Buffer** and centrifuge. Discard the flow-through.

12. Add 400 **RNA Wash Buffer** and centrifuge the column for 1 minute to ensure complete removal of the wash buffer. Carefully transfer the column into a new nuclease-free tube (not provided).

13. Add 100 µl **ZymoBIOMICS™ DNase/RNase-Free Water** directly to the column matrix and then centrifuge to elute.

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

14. Place a **Zymo-Spin™ III-HRC Filter** in a new **Collection Tube** and add 600 µl **ZymoBIOMICS™ Prep Solution**. Centrifuge at 8,000 x *g* for 3 minutes.

15. Transfer the eluted RNA (step 13) 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 RNA can be used immediately or stored frozen.

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 ≥ 1 month or long term at frozen temperature.

Ordering Information

Product Description	Catalog No.	Size
ZymoBIOMICS™ RNA Miniprep Kit	R2001	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
RNA Lysis Buffer	R1060-1-50 R1060-1-100	50 ml 100 ml
RNA Prep Buffer	R1060-2-25 R1060-2-100	25 ml 100 ml
RNA Wash Buffer (concentrate)	R1003-3-24 R1003-3-48	24 ml 48 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 prep
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 RNA	
MagBeads #R2137, R2138	Automatable (Tecan, Hamilton, Kingfisher, etc.)

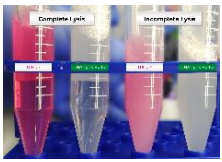
✓ For RNA clean-up (purification) from the aqueous phase (e.g., TRIzol, TRI Reagent or similar) or from any enzymatic reaction (e.g., DNase I treated RNA):

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 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 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 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

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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.

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