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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Product Contents

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.

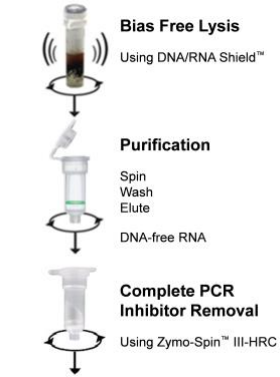
Product Description

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.

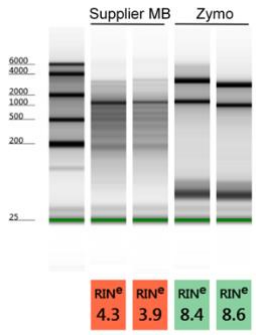
The **ZymoBIOMICS™** innovative lysis system eliminates bias associated with unequal lysis efficiencies of different organisms (e.g. gram negative/positive bacteria, fungus, protozoans, and algae). The provided **DNA/RNA Shield™** preserves nucleic acids at ambient temperatures, providing an unbiased 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.

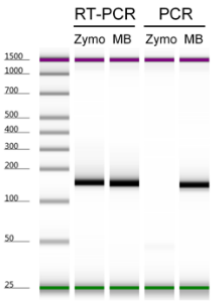
High-Quality RNA



***Ultra-pure* Total 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™**, 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 **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., 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 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 (1:1) and mix.

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.

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

9. Transfer the sample 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.

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

(continue to purification, page 7)

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1 To process samples > 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.

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 ≥ 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.)

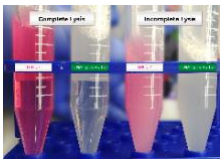
✓ For RNA clean-up (purification) from the aqueous phase (e.g., TRIzol, TRI Reagent or similar) or from any enzymatic reaction (e.g., DNase I treated RNA):

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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