ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZymoBIOMICS™ RNA Miniprep Kit

Catalog No. **R2001**

Highlights

- Rapid, robust, and simple purification of high quality, inhibitor-free total RNA (including small/micro RNAs) from any sample including feces, soil, water, biofilms, swabs, saliva, and body fluids, *etc.*
- **ZymoBIOMICS™** innovative lysis system enables efficient and unbiased lysis of microbes including gram positive/negative bacteria, fungus, protozoans, algae, viruses, *etc*.
- *DNA-free* RNA is ready for use in any downstream application. *DNase I included.*

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ZYMO RESEARCH CORP.

Phone: (949) 679-1190 ▪ Toll Free: (888) 882-9682 ▪ Fax: (949) 266-9452 ▪ info@zymoresearch.com ▪ www.zymoresearch.com

Satisfaction of all Zymo Research products is guaranteed. If you are dissatisfied with this product please call 1-888-882-9682.

For assistance, contact us at tech@zymoresearch.com.

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

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.

Product Contents

ZymoBIOMICS™ RNA Miniprep Kit (Kit Size)	**R2001** (50 Preps.)	**Storage Temperature**
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	50	Room Temp.
DNA/RNA Shield™	50 ml	Room Temp.
RNA Lysis Buffer	50 ml	Room Temp.
RNA Prep Buffer	2x 25 ml	Room Temp.
RNA Wash Buffer[1] (concentrate)	24 ml	Room Temp.
DNase/RNase-Free Water	30 ml	Room Temp.
ZymoBIOMICS™ HRC Prep Solution	30 ml	Room Temp.
DNase I[2] (lyophilized)	1	Room Temp.
DNA Digestion Buffer	4 ml	Room Temp.
Zymo-Spin™ III-HRC Filters	50	Room Temp.
Zymo-Spin™ IIICG Columns	100	Room Temp.
Collection Tubes	150	Room Temp.
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Note - 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.

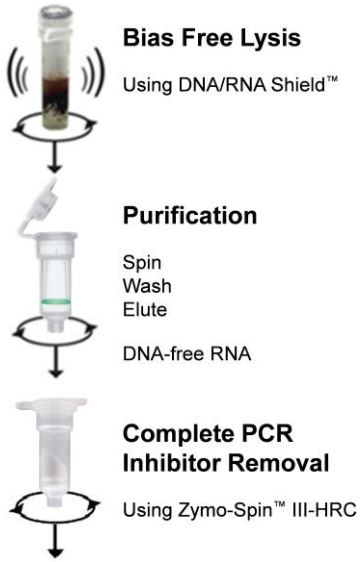
[1] Before starting, add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.
[2] Prior to use, reconstitute the lyophilized **DNase I** with 275 µl **DNase/RNase-Free Water**. Mix by gentle inversion. Store aliquots at -20ºC.

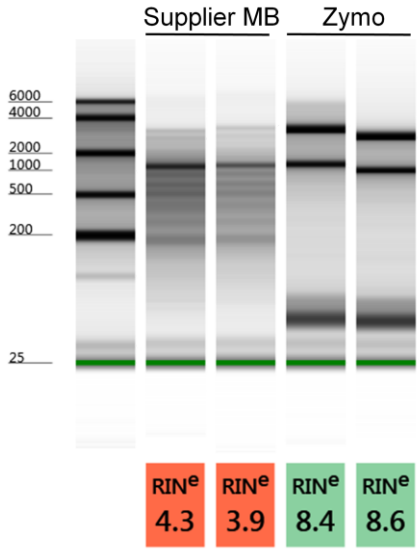
Specifications

- **Sample Types** – Bacterial, fungal, protozoan, algae, viral, mitochondrial, and host RNA is efficiently isolated from ≤ 200 mg of mammalian feces, ≤ 250 mg soil, ≤ 200 mg plant/seed, 50-100 mg (wet weight) fungal bacterial cells[1], biofilms, water, and swabs.
- **Bead beating system** – **ZymoBIOMICS™** innovative lysis system ensures complete homogenization of microbial cell walls and accurate microbial RNA 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.
- **RNA Size** – RNAs ≥17 nucleotides.
- **RNA Purity** – A_{260}/A_{280} >1.8, A_{260}/A_{230} >1.8. DNase I provided for removal of DNA.
- **Yield** – The RNA binding capacity of the **Zymo-Spin™ IIICG Column** is ~100 µg.
- **RNA Storage** – RNA eluted with **DNase/RNase-Free Water** can be stored at ≤-70ºC. The addition of RNase inhibitors in highly recommended for prolonged storage.
- **Required Equipment** – Microcentrifuge, vortex, cell disrupter (recommended).

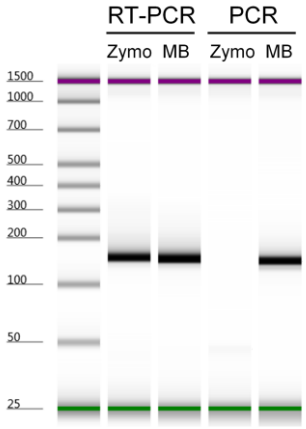
Product Description

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 RNAs 17-200 nt)* that is free of PCR inhibitors (*e.g.* polyphenols, humic acids, and fulvic acids) and is ready for RT-PCR, hybridization, sequencing, *etc*.

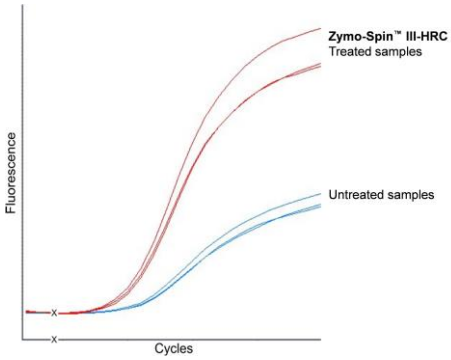




Human stool RNA isolated with the **ZymoBIOMICS™ RNA Miniprep Kit** is higher quality (right); compared to Supplier MB procedures (left). Quality assessed by Agilent 2200 TapeStation™.



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™.



Total RNA was isolated with or without inclusion of the **Zymo-Spin™ III-HRC Filter**. Earlier amplification cycles indicate complete removal of PCR inhibitors.

For **Assistance**, please contact Zymo Research Technical Support at 1-888-882-9682 or e-mail tech@zymoresearch.com.

Ensure the RNA isolation procedure is performed in an RNase-free environment.

The lyophilized **DNase I** is stable as shipped.

Notes:

[1] DNA/RNA Shield™ Lysis Tube w/ Swab (Microbe) Cat. No. R1104

[2] For water samples, filter using desired filter (not provided). Cut the filter into small pieces and place into ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm).

Swabs can also be cut or broken and placed directly in bead beating tube.

[3] DNA/RNA Shield™ Fecal Collection Tube Cat. No. R1101

DNA/RNA Shield™ Collection Tube w/ Swab (1 or 2 ml fill) Cat. Nos. R1107, R1109

Reagent Preparation

- ✓ Before starting, add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.
- ✓ Add 275 µl **DNase/RNase-Free Water** per vial to reconstitute the lyophilized **DNase I** at 1 U/µl. Mix by gentle inversion. Store frozen aliquots at -20ºC.

Protocols

The RNA isolation consists of two steps: (I) Sample Preparation & (II) RNA Purification.

Sample Preparation

All centrifugation steps should be performed at 10,000 - 16,000 *x g* for 30 seconds unless specified.
All steps should be performed at room temperature (20-30ºC) unless specified.

1. Add sample to a **ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)**; (S6012-50; available separately). Add 750 µl **DNA/RNA Shield™** to the tube and cap tightly to assure no leakage during bead beating. If sample is already collected using **DNA/RNA Shield – Lysis Tube (Microbe)**[1], proceed to Step 2 directly instead.

Sample Type	Maximum Input
Feces	200 mg
Soil	250 mg
Plant/Seed	200 mg
Liquid Samples and Swab Collections[2]	250 µl
Cells (Suspended in DNA/RNA Shield™ or isotonic buffer *e.g.* PBS)	50-100 mg (wet weight) (10^9 bacterial, 10^8 yeast cells, 10^7 mammalian cells)
DNA/RNA Shield™ Collection Devices[3] (Cat Nos. R1101, R1107, R1109)	750 µl

2. Secure in a bead beater fitted with a 2 ml tube holder assembly and process at maximum speed for ≥ 5 minutes.

   Processing time will vary based on sample input and bead beater. Times may be as little as 5 minutes when using high-speed cell disrupters (FastPrep®-24) or as long as 20 minutes when using lower speeds (e.g. Disruptor Genie™).

3. Centrifuge the **ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)** in a microcentrifuge for 1 minute.

4. Transfer up to 400 µl supernatant to a new RNase-free tube (not provided). Proceed to RNA Purification.

RNA Purification

All centrifugation steps should be performed at 10,000 - 16,000 *x g* for 30 seconds unless specified.
All steps should be performed at room temperature (20-30ºC) unless specified.

1. Add 2 volumes of **RNA Lysis Buffer** to the sample and mix.
2. Add an equal volume of ethanol (95-100%) and mix.
3. Transfer the mixture into a **Zymo-Spin™ IIICG Column**[1] in a **Collection Tube** and centrifuge. Discard the flow-through.
4. Add 400 µl **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
5. Add 400 µl **RNA Wash Buffer** to the column and centrifuge. Transfer the column carefully into an RNase-free tube (not provided).
6. Add 85 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge.
7. Add 10 µl **DNA Digestion Buffer** and 5 µl **DNase I**[2] to the sample and mix gently. Incubate at room temperature (20-30ºC) for 15 minutes.
8. Add 2 volumes of **RNA Lysis Buffer** to the sample and mix.
9. Add an equal volume of ethanol (95-100%) and mix.
10. Transfer the sample to a new **Zymo-Spin™ IIICG Column** in a **Collection Tube** and centrifuge. Discard the flow-through.
11. Add 400 µl **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
12. Add 700 µl **RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.
13. Add 400 µl **RNA Wash Buffer** and centrifuge the column for 2 minutes to ensure complete removal of the wash buffer. Transfer the column carefully into an RNase-free tube (not provided).
14. Add 100 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

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

    The filtered RNA can be used immediately or stored at ≤-70ºC.

Notes:

[1] To process samples >800 µl, **Zymo-Spin™** columns may be reloaded.

[2] Prior to use, reconstitute the lyophilized **DNase I** as indicated on the vial. Store frozen aliquots.

Ordering Information

Product Description	Kit Size	Catalog No.
ZymoBIOMICS™ RNA Miniprep Kit	50 Preps.	R2001
ZymoBIOMICS™ DNA/RNA Miniprep Kit	50 Preps.	R2002

For Individual Sale	Amount	Catalog No.
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	50	S6012-50
DNA/RNA Shield™	50 ml 250 ml	R1100-50 R1100-250
RNA Lysis Buffer	50 ml 100 ml	R1060-1-50 R1060-1-100
RNA Prep Buffer	10 ml 25 ml 100 ml	R1060-2-10 R1060-2-25 R1060-2-100
RNA Wash Buffer (concentrate)	6 ml 12 ml 24 ml 48 ml	R1003-3-6 R1003-3-12 R1003-3-24 R1003-3-48
DNase/RNase-Free Water	1 ml 4 ml 6 ml 10 ml 30 ml	W1001-1 W1001-4 W1001-6 W1001-10 W1001-30
DNase I Set (lyophilized) DNase I (250 U) & DNA Digestion Buffer (4 ml)	1 set	E1010
OneStep™ PCR Inhibitor Removal Kit	50	D6030
Zymo-Spin™ IIICG Columns	50	C1006-50-G
Collection Tubes	50 500 1000	C1001-50 C1001-500 C1001-1000
DNA/RNA Shield™ - Fecal Collection Tube	10	R1101
DNA/RNA Shield™ - Collection Tube w/ Swab	10 50	R1106 R1107
DNA/RNA Shield™ - Lysis Tube (Microbe)	50	R1103

RNA MADE SIMPLE

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