ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZymoBIOMICS™ RNA Mini 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.

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Satisfaction of all Zymo Research products is guaranteed. If you are dissatisfied with this product please contact us.

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.

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

Product Contents

ZymoBIOMICS™ RNA Mini Kit (Kit Size)	**R2001** (50 Preps.)	**Storage Temperature**
DNA/RNA Shield™ - Lysis Tube (Microbe)	50	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.
DNase I[2] (lyophilized)	1	Room Temp.
DNA Digestion Buffer	4 ml	Room Temp.
Zymo-Spin™ IV-HRC Spin Filters (green tops)	50	Room Temp.
Zymo-Spin™ IIICG Columns	100	Room Temp.
Collection Tubes	150	Room Temp.
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Storage Temperature - Store all kit components (*i.e.,* buffers, columns) at room temperature.

[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.

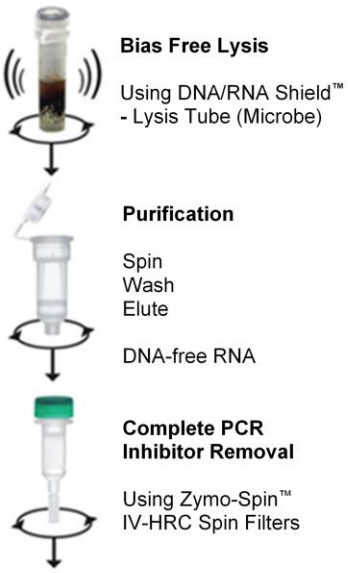
Specifications

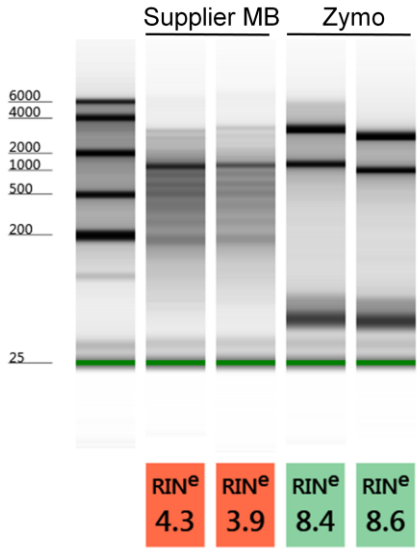
- **Sample Sources** – Bacterial, fungal, protozoan, algae, viral, mitochondrial, and host RNA is efficiently isolated from ≤ 200 mg of mammalian feces, ≤ 250 mg soil, ≤ 200 mg plant/seed, 50-100 mg (wet weight) fungal bacterial cells[1], biofilms, and water.
- **Bead beating system** – **ZymoBIOMICS™** innovative lysis system ensures complete homogenization of the 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 safe 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 complete removal of DNA.
- **RNA Recovery** – The RNA binding capacity of the **Zymo-Spin™ IIICG Column** is ~100 µg.
- **RNA Storage** – RNA eluted with **DNase/RNase-Free Water** (provided) can be stored at ≤70ºC. The addition of RNase inhibitors in highly recommended for prolonged storage.
- **Equipment Needed** – Microcentrifuge, vortex, cell disrupter (recommended).

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

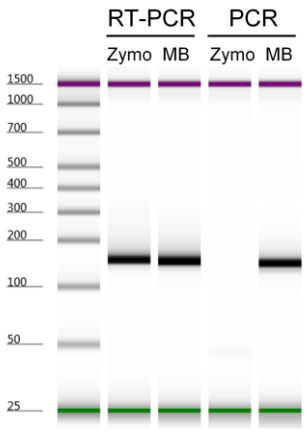
Product Description

The **ZymoBIOMICS™ RNA Mini 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*.

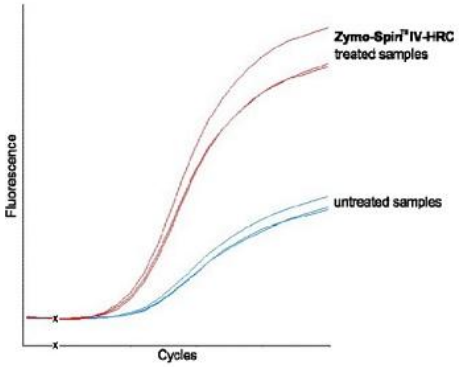




Human stool RNA isolated with the **ZymoBIOMICS™ RNA Mini Kit** is higher quality (right); compared to Supplier MB procedures (left). Quality assessed by Agilent 2200 TapeStation.



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 Mini Kit** indicates DNA-free RNA. Quality assessed by Agilent 2200 TapeStation.



Total RNA isolated from human stool with or without inclusion of the Zymo-Spin™ IV-HRC Spin Filter during the **ZymoBIOMICS™ RNA Mini Kit** protocol. 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.

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 **DNA/RNA Shield™ - Lysis Tube (Microbe)**. Cap tightly to assure no leakage during bead beating.

Sample Type	Maximum Input
Feces	200 mg
Soil	250 mg
Plant/Seed	200 mg
Liquid Samples and Water[1]	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)

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 **DNA/RNA Shield™ - Lysis Tube (Microbe)** 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.

Notes:

[1] For water samples, filter using desired filter (not provided). Cut the filter into small pieces and place into DNA/RNA Shield™ - Lysis Tube (Microbe).

RNA Purification

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 **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. Preparing **Zymo-Spin™ IV-HRC Spin Filter** (green tops)

    a. Snap off the base of the filter and place into a Collection Tube. Centrifuge at 8,000 *x g* for 3 minutes. Discard the flow-through.

    b. Remove the cap and add 400 µl **DNase/RNase-Free Water** to the filter. Loosely cap the filter and centrifuge at 8,000 *x g* for 2 minutes.
16. Transfer the eluted RNA (step 13) into a prepared **Zymo-Spin™ IV-HRC Spin Filter** in an RNase-free tube (not provided) and centrifuge at 8,000 *x g* for 1 minute.

    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	Catalog No.	Kit Size
ZymoBIOMICS™ RNA Mini Kit	R2001	50 Preps.

For Individual Sale	Catalog No.	Amount
DNA/RNA Shield™ - Lysis Tube (Microbe)	R1100-1-B15	50
RNA Lysis Buffer	R1060-1-50 R1060-1-100	50 ml 100 ml
RNA Prep Buffer	R1060-2-10 R1060-2-25 R1060-2-100	10 ml 25 ml 100 ml
RNA Wash Buffer (concentrate)	R1003-3-6 R1003-3-12 R1003-3-24 R1003-3-48	6 ml 12 ml 24 ml 48 ml
DNase/RNase-Free Water	W1001-1 W1001-4 W1001-6 W1001-10 W1001-30	1 ml 4 ml 6 ml 10 ml 30 ml
DNase I Set (lyophilized) DNase I (250 U) & DNA Digestion Buffer (4 ml)	E1010	1 set
Zymo-Spin™ IV-HRC Spin Filters (green tops)	C1010-50	50
Zymo-Spin™ IIICG Columns	C1006-50-G	50
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1000
DNA/RNA Shield™ - Fecal Collection Tube	R1100-9-T	10 tubes
DNA/RNA Shield™ - Collection Tube	R1100-1-T	50
DNA/RNA Shield™	R1100-50 R1100-250	50 ml 250 ml
DNA/RNA Shield™ (2X concentrate)	R1200-25 R1200-125	25 ml 125 ml

RNA MADE SIMPLE

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