

INSTRUCTION MANUAL

ZymoBIOMICS™ MagBead RNA

Catalog Nos. R2137, R2138

Highlights

- **Unbiased Lysis:** 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.
- **Ultra-Pure:** High-quality total RNA (including small/microRNAs) is inhibitor-free and ready for RT-qPCR and microbiome measurements using Next-Gen Sequencing.
- **High-Sensitivity:** Increased detection limit of very low abundance organisms.

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

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

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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Product Contents

ZymoBIOMICS™ MagBead RNA (Kit Size)	R2137 (96 Preps)	R2138 (4 x 96 Preps)	Storage Temperature
ZymoBIOMICS™ MagBinding Beads	6 ml	2 x 12 ml	Room Temp.
DNA/RNA Shield™	2 x 50 ml	2 x 250 ml	Room Temp.
RNA Lysis Buffer	50 ml	2 x 100 ml	Room Temp.
RNA Prep Buffer	50 ml	2 x 100 ml	Room Temp.
MagBead DNA/RNA Wash 1¹ (concentrate)	2 x 30 ml	2 x 120 ml	Room Temp.
MagBead DNA/RNA Wash 2² (concentrate)	2 x 20 ml	2 x 80 ml	Room Temp.
ZymoBIOMICS™ DNase/RNase-Free Water	30 ml	2 x 50 ml	Room Temp.
DNase I³ (lyophilized)	3 x 250 U	2 x 1500 U	Room Temp. (-20°C; reconstituted)
DNA Digestion Buffer	4 ml	4 ml	Room Temp.
Proteinase K³ (lyophilized) & Storage Buffer	20 mg	4 x 20 mg	Room Temp. (-20°C; reconstituted)
Instruction Manual	1	1	-
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)		Available separately (S6012-50)	
ZR-96 BashingBead™ Lysis Rack (0.1 & 0.5 mm)		Available separately (S6002-96-3)	

¹ Add 20 ml (R2137) or 80 ml (R2138) of isopropanol to the **MagBead DNA/RNA Wash 1** concentrate.

² Add 30 ml (R2137) or 120 ml (R2138) of isopropanol to the **MagBead DNA/RNA Wash 2** concentrate.

³ Prior to use, reconstitute lyophilized **DNase I** and lyophilized **Proteinase K** according to page 3, Reagent Preparation.

Specifications

- **Sample Types** – Bacterial, fungal, protozoan, algae, viral, mitochondrial RNA are efficiently isolated from ≤ 50 mg of mammalian feces, ≤ 50 mg soil, and 5-20 mg (wet weight) of fungal/bacterial cells, biofilms, water, and swabs.
- **Sample Preservation** – **DNA/RNA Shield™** lyses cells, inactivates nucleases and infectious agents and is ideal for safe sample storage and transport at ambient temperatures.
- **Size Limits** – Total RNA including small/microRNAs ≥17 nt.
- **Purity** – High-quality RNA is ready for Next-Gen Sequencing, RT/PCR, hybridization, etc.
- **Binding Capacity** – 15 µg total RNA per 30 µl **ZymoBIOMICS™ MagBinding Beads**.
- **Storage** – RNA eluted with **ZymoBIOMICS™ DNase/RNase-Free Water** (provided) can be stored frozen. The addition of RNase inhibitors is highly recommended for prolonged storage.
- **Equipment Needed** – Magnetic stand, heat block, liquid handler or robotic sample processor (user provided).
- **Recommended Materials** (sold separately) – 96-well Collection Plate (C2002; capacity is up to 1.2 ml/well), 96-Well Block (P1001; capacity is up to 2 ml/well), 96-well Elution Plate (C2003), Cover Foil (C2007), ZR-96 MagStand (P1005).

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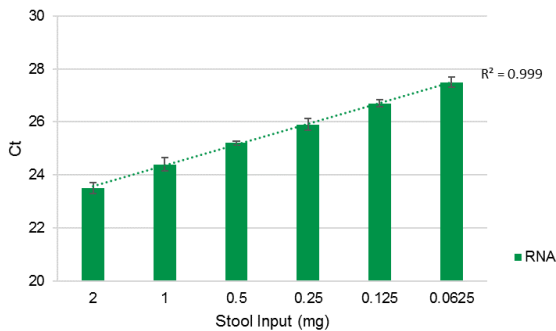
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Product Description

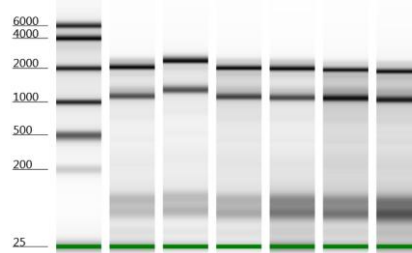
The **ZymoBIOMICS™ MagBead RNA** kit provides a high-throughput, magnetic bead-based purification of total RNA (including small/microRNAs) from any sample source (e.g., microbes including gram positive/negative bacteria, fungi, protozoans, viruses, feces, soil, plant, water, biofilms, swabs, saliva, body fluids and *etc.*). The provided **DNA/RNA Shield™** inactivates infectious agents and is ideal for sample storage at ambient temperatures. Total RNA is eluted into $\geq 50 \mu\text{l}$ of **ZymoBIOMICS™ DNase/RNase-Free Water** and is ready for any downstream application including Next-Gen Sequencing, RT-qPCR, *etc.*

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

High-Quality RNA

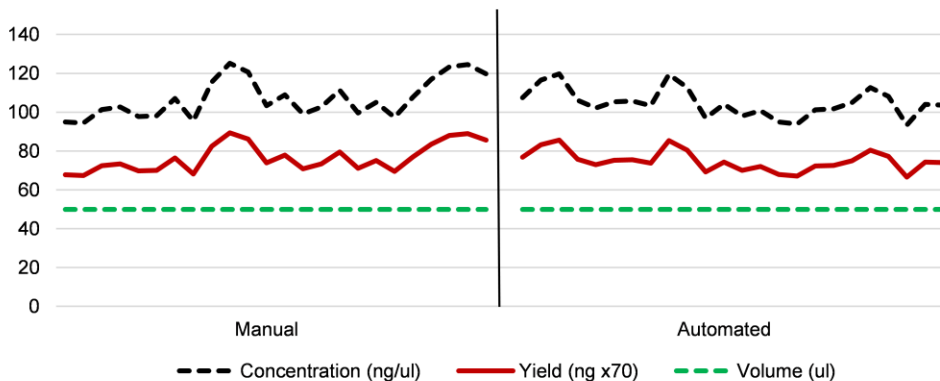


Human stool RNA is purified with the **ZymoBIOMICS™ MagBead RNA** kit from low biomass inputs. Results analyzed by RT-qPCR (n=2).



Total RNA from human stool is high-quality using the **ZymoBIOMICS™ MagBead RNA** kit (Agilent 2200 TapeStation).

Reproducible Sample Processing



Concentration, yield, and elution volume across replicate samples extracted with the **ZR BashingBead™ Lysis Tubes** and **ZymoBIOMICS™ MagBead RNA** kit are reproducible and consistent. Total nucleic acids were purified from human stool (~40 mg/well).

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The lyophilized **Proteinase K** and **DNase I** are stable as shipped.

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

Notes:

¹ At this point, samples in DNA/RNA Shield can be stored at ambient temperature (4-25°C) for a month, 3 days at 37°C, or long-term (> 1 year) -20°C or below.

² For water samples, filter using desired filter (not provided). Cut the filter into small pieces and place into ZR BashingBead™ Lysis Tubes or into 96-well Lysis Rack.

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

³ Secure in a high-speed bead beater and process at maximum speed for ≤ 5 minutes (e.g., Bertin Precellys, FastPrep®) or for ≥ 10 minutes when using low speed homogenizers (e.g., Disruptor Genie™).

Reagent Preparation

- ✓ Add 20 ml (R2137) or 80 ml (R2138) isopropanol to the **MagBead DNA/RNA Wash 1** concentrate.
- ✓ Add 30 ml (R2137) or 120 ml (R2138) isopropanol to the **MagBead DNA/RNA Wash 2** concentrate.
- ✓ Add 1.2 ml **Proteinase K Storage Buffer** per vial to reconstitute the lyophilized **Proteinase K** at 20 mg/ml. Vortex to dissolve. Store frozen.

Prep Size	DNase I (lyophilized)	ZymoBIOMICS™ DNase/RNase-Free Water	DNA Digestion Buffer
96 preps	3 x 250 U	6.75 ml	0.75 ml
4 x 96 preps	2 x 1500 U	27 ml	3 ml

- a. Reconstitute **DNase I** with **ZymoBIOMICS™ DNase/RNase-Free Water** (table above), transfer into an RNase-free tube (e.g., 15 ml conical tube; not provided) and mix by inversion. Store frozen aliquots.
 - b. Add **DNA Digestion Buffer** to the reconstituted DNase I (table above) and mix by inversion, then place on ice until ready to use. Add 50 µl **DNase I Reaction Mix** per sample during RNA Purification, page 4.
- ✓ Prepare **DNase I Reaction Mix** (according to the example below; scale as needed).

Protocol

The isolation consists of: (I) Sample Preparation and (II) RNA Purification

(I) Sample Preparation

All centrifugation steps should be performed at 10,000 - 16,000 x g for 30 seconds, unless specified. For 96-well lysis rack, centrifuge at 4,000 x g for 5 minutes. All steps should be performed at room temperature (20-30°C), unless specified.

1. Add 750 µl **DNA/RNA Shield™** to a sample (see table below) and mix and/or homogenize¹.

Sample Type	Maximum Input
Feces	50 mg
Soil	50 mg
Liquid Samples ² and Swab Collections	250 µl
Cells (resuspended in DNA/RNA Shield™ or isotonic buffer, e.g. PBS)	5-20 mg (wet weight; 2x10 ⁸ bacterial, 2x10 ⁷ yeast cells, 2x10 ⁶ mammalian cells)
Samples in DNA/RNA Shield™ (10% v/v)	250

2. To achieve unbiased lysis of different organisms, including hard-to-lyse microbes, perform mechanical homogenization³ (Recommended e.g., **lysis tubes S6012-50** or **lysis rack S6002-96-3**; each sold separately). Then centrifuge to pellet debris and transfer 200 µl supernatant to a new tube.
3. Add 10 µl **Proteinase K** for every 200 µl sample. Mix and incubate at room temperature (20-30°C) for 30 minutes.
4. Proceed to RNA Purification, page 4.

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(II) RNA Purification

1. Add 200 µl (1 volume) **RNA Lysis Buffer** to 200 µl sample and mix well¹.
2. Add 400 µl ethanol (95-100%) to the sample and mix well¹.
3. Add 30 µl **ZymoBIOMICS™ MagBinding Beads** and mix well¹ for 20 minutes.

Important: **ZymoBIOMICS™ MagBinding Beads** settle quickly, ensure that beads are kept in suspension while dispensing.
4. Transfer the plate/tube to the magnetic stand² until beads have pelleted, then aspirate³ and discard the cleared supernatant.
5. Add 500 µl **MagBead DNA/RNA Wash 1** and mix well¹. Pellet the beads^{2,3} and discard the supernatant.
6. Add 500 µl **MagBead DNA/RNA Wash 2** and mix well¹. Pellet the beads^{2,3} and discard the supernatant.
7. Add 500 µl ethanol (95-100%) and mix well¹. Pellet the beads^{2,3} and discard the supernatant.
8. Repeat step 7.

9. **DNase I** treatment (optional)

- (D1) Add 50 µl **DNase I Reaction Mix** and mix gently for 10 minutes.
- (D2) Add 500 µl **DNA/RNA Prep Buffer** and mix well¹ for 10 minutes. Pellet the beads^{2,3} and discard the supernatant.
- (D3) Repeat steps 7-8.

10. Dry the beads for 10 minutes or until dry⁴.
11. To elute RNA from the beads, add 50 µl **ZymoBIOMICS™ DNase/RNase-Free Water** and mix well¹ for 5 minutes.
12. Transfer the plate/tube to the magnetic stand² until beads have pelleted, then aspirate³ and dispense the eluted DNA/RNA to a new plate/tube.

The eluted RNA can be used immediately or stored frozen.

Perform all steps at **room temperature**, unless specified.

Notes:

¹ For all buffer additions and incubation steps, **mix well** by pipetting the beads up and down several times and/or by shaking (vortexing) at ~1,300 rpm. Optimization may be required.

² Use a strong-field magnetic stand or separator (e.g., ZR-96 MagStand, P1005; sold separately) until beads have pelleted.

³ Some beads will adhere to the sides of the well. When removing the supernatant, aspirate slowly to allow these beads to be pulled to the magnet as the liquid level is lowered.

⁴ Beads will change in appearance from glossy black when still wet to a dull brown when fully dry. Alternatively, a heat block can be used (25-55°C).

For assistance with automating/scripting this workflow onto your platform or device, contact our automation specialists at automation@zymoresearch.com

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
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Appendix A: Compatible DNA/RNA Shield™ Collection Devices

DNA/RNA Shield™

Sample Collection and Preservation



Blood	Swab	Stool	Tissue
Fresh EDTA Citrate Heparin	Mouth Nose Throat Fluid	Virus Microbe Host	Animal Plant Insect Microbe
			
16x100 mm vacuum tube 3 ml draw R1150 (50 pack)	12x80 mm tube + HydraFlock swab 1 ml reagent R1106 (10 pack) R1107 (50 pack) 2 ml reagent R1108 (10 pack) R1109 (50 pack)	20x76 mm scoop tube 9 ml reagent R1101 (10 pack)	2 ml lysis tube 1 ml reagent R1102 (50 pack) Microbe (+beads) R1103 (50 pack) Microbe w/ swab R1104 (50 pack) Tissue R1105 (50 pack)

Appendix B: Sample Preservation in DNA/RNA Shield™

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.
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 (4-30°C) for a month, 3 days at 37°C or long term (>1 year) at -20°C or below.

Automation Scripts

The ZymoBIOMICS™ MagBead RNA (R2137/R2138) is compatible with automated platforms. For automation scripts and related technical support, email automation@zymoresearch.com. In the subject line, please include "Automation Scripts", instrument used and the product catalog number.

Troubleshooting

For **Technical Assistance**, please contact 1-888-882-9682 or E-mail tech@zymoresearch.com.

Problem	Possible Causes and Suggested Solutions
Low Recovery	
<i>Binding Conditions</i>	<ul style="list-style-type: none"> • Increase Binding Time: At all binding steps, increase binding time for an additional ≥ 10 minutes (e.g., 30 minutes). Depending on the amount of biomass, more time may be required to allow DNA and RNA to be sufficiently bound to beads. • Shaking/Mixing: Mix well by pipetting up and down several times and/or by shaking (vortexing) at high speed. Make sure that the beads are resuspended throughout the bind, wash and elution steps. • Magnetic Beads: ZymoBIOMICS™ MagBinding Beads settle quickly, ensure that beads are kept in suspension while dispensing. • Adjust Reagent Volumes: Low recovery may be due to input (high biomass) usually indicative by a viscous, cloudy lysate. Increase or titrate the volume of DNA/RNA Lysis Buffer, magnetic beads and elution volume proportionally.
<i>Elution Parameters</i>	<ul style="list-style-type: none"> • Elution Volume: Elution volume can be increased to ensure adequate recovery (e.g., $\geq 50 \mu\text{l}$). • Adjust Temperature: Increase elution temperature to 55°C. This can improve recovery if beads are “sticky” (for high molecular weight genomic DNA, etc.).
Low Purity ($A_{260/230 \text{ nm}}$)	
<i>Washing of Beads</i>	<ul style="list-style-type: none"> • Shaking/Mixing: Mix well by pipetting up and down several times and/or by shaking (vortexing) at high speed. Make sure that the beads are resuspended throughout the bind, wash and elution steps.
<i>Drying of Beads</i>	<ul style="list-style-type: none"> • Adjust Drying Parameters: Increase the time for drying the beads (≥ 10 minutes) at room temperature. Alternatively, a heat block can be used (55°C).
Degraded Nucleic Acids	
<i>Sample Input</i>	<ul style="list-style-type: none"> • Sample Preservation: Check initial sample by establishing kit controls with a known quality and concentration to eliminate artifacts originating from kit to kit variation. Make sure to transfer samples to provided DNA/RNA Shield™ to ensure sample stabilization and minimize degradation effects.
<i>Bead-beating</i>	<ul style="list-style-type: none"> • Sample Lysis: Bead-beating times may need to be optimized to ensure sufficient lysis without compromising sample quality. These exact settings can vary from low to high-speed cell disrupters. Bead-beating will shear genomic DNA to some extent. For more information bead-beating, read Zymo Research’s article, “The Lysis Bias Crisis”.

Ordering Information

Product Description	Catalog No.	Kit Size
ZymoBIOMICS™ MagBead RNA	R2137 R2138	96 Preps. 4 x 96 Preps.

For Individual Sale	Catalog No.	Amount
ZymoBIOMICS™ MagBinding Beads	D4302-6-6 D4302-6-12	6 ml 12 ml
DNA/RNA Shield™	R1100-50 R1100-250	50 ml 250 ml
RNA Lysis Buffer	R1060-1-50 R1060-1-100 R1060-1-200	50 ml 100 ml 200 ml
RNA Prep Buffer	R1060-2-50 R1060-2-100	50 ml 100 ml
MagBead DNA/RNA Wash 1	R2130-1-30 R2130-1-120	30 ml 120 ml
MagBead DNA/RNA Wash 2	R2130-2-20 R2130-2-80	20 ml 80 ml
ZymoBIOMICS™ DNase/RNase-Free Water	D4302-5-1 D4302-5-10 D4302-5-30 D4302-5-50	1 ml 10 ml 30 ml 50 ml
DNase I Set (lyophilized) DNase I (250 U) & DNA Digestion Buffer (4 ml)	E1010	1 set
Proteinase K (lyophilized) (supplied with Proteinase K Storage Buffer)	D3001-2-5 D3001-2-20	5 mg set 20 mg set
ZR BashingBead™ Lysis Rack (0.1 & 0.5 mm)	S6002-96-3	2 Racks
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	S6012-50	50 Tubes

Sample Collection	Amount	Catalog No.
DNA/RNA Shield™ - Fecal Collection Tube	10	R1101
DNA/RNA Shield™ - Collection Tube	50	R1102
DNA/RNA Shield™ - Lysis Tube (Microbe)	50	R1103
DNA/RNA Shield™ - Lysis Tube w/ Swab (Microbe)	50	R1104
DNA/RNA Shield™ - Collection Tube w/ Swab	10 50	R1106 R1107

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