ZYMO RESEARCH

RNA Purification Made Simple™

ZymoBIOMICS™ MagBead DNA/RNA

Microbiome DNA and RNA from any sample

Highlights

- 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.
- High-throughput, magnetic bead-based purification of high-quality DNA/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:
R2135, R2136

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

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682

Table of Contents

Product Contents

ZymoBIOMICS™ MagBead DNA/RNA	R2135 (96 prep)	R2136 (4 x 96 prep)
ZymoBIOMICS™ MagBinding Beads	6 ml	12 ml (x2)
DNA/RNA Shield™	50 ml (x2)	250 ml (x2)
DNA/RNA Lysis Buffer	50 ml (x2)	200 ml (x2)
DNA/RNA Prep Buffer	50 ml (x2)	200 ml (x2)
MagBead DNA/RNA Wash 1[1] (concentrate)	30 ml (x3)	120 ml (x3)
MagBead DNA/RNA Wash 2[2] (concentrate)	20 ml (x3)	80 ml (x3)
ZymoBIOMICS™ DNase/RNase-Free Water	30 ml	50 ml (x2)
DNase I[3] (lyophilized)	250 U (x3)	1500 U (x2)
DNA Digestion Buffer	4 ml	4 ml
Proteinase K[3] (lyophilized) & Storage Buffer	20 mg	20 mg (x4)
Instruction Manual	1	1
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	Available separately (S6012-50)	
ZymoBIOMICS BashingBead™ Lysis Rack (0.1 & 0.5 mm)	Available separately (S6002-96-7)	

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature.
Before use:
1 Add 20 ml (R2135) or 80 ml (R2136) of isopropanol to the **MagBead DNA/RNA Wash 1** concentrate.
2 Add 30 ml (R2135) or 120 ml (R2136) of isopropanol to the **MagBead DNA/RNA Wash 2** concentrate.
3 Reconstitute the lyophilized **Proteinase K** and **DNase I** according to Buffer Preparation, page 4.

Specifications

- **Sample Sources –** Bacterial, fungal, protozoan, algae, viral, mitochondrial, and host DNA and RNA are efficiently isolated from ≤ 50 mg of soil, mammalian feces and plant/seed, 5-20 mg (wet weight[1]) of fungal/bacterial cells, 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. Lysis tubes (S6012-50) and 96-well lysis rack (S6002-96-7) available separately).

- **Sample Preservation – DNA/RNA Shield™** lyses cells, inactivates nucleases and infectious agents, and is ideal for sample storage and transport at ambient temperatures.

- **Size –** DNA and total RNA including small/microRNAs (≥ 17 nt).

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

- **Binding Capacity –** 15 µg DNA/RNA per 30 µl **ZymoBIOMICS™ MagBinding Beads**.

- **Elution Volume –** 50 µl **ZymoBIOMICS™ DNase/RNase-Free Water**.

- **Equipment Needed** (user provided) **–** Magnetic stand or separator, heat block, liquid handler or robotic sample processor.

- **Recommended Materials** (available 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).

  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

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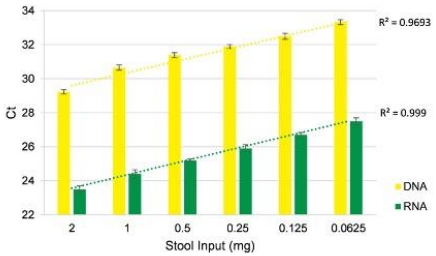
1 This equates to approximately $2x10^8$ bacterial cells, $2x10^7$ yeast cells, and $2x10^6$ mammalian cells.

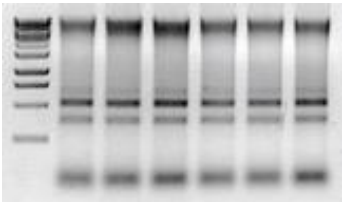
Product Description

The **ZymoBIOMICS™ MagBead DNA/RNA** kit provides a high-throughput, magnetic bead-based purification of both high-quality DNA and total RNA (including small/microRNAs) from the same starting sample. The provided **DNA/RNA Shield™** inactivates infectious agents and is ideal for sample storage at ambient temperatures. The extraction method utilizes magnetic beads for DNA/RNA extraction without the use of phenol and is eluted into ≥ 50 µl of **ZymoBIOMICS™ DNase/RNase-Free Water**. DNA/RNA is ready for any downstream application including Next-Gen Sequencing, RT-qPCR, etc.

High-Quality DNA and RNA

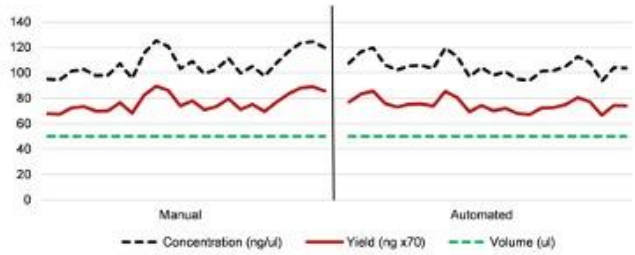


ZymoBIOMICS™ MagBead DNA/RNA kit is able to purify DNA (top; yellow) and RNA (bottom; green) from human stool (at low biomass inputs). Results analyzed by qPCR (n=2).



DNA & RNA from human stool are high-quality using the **ZymoBIOMICS™ MagBead DNA/RNA** kit. Final eluate were analyzed on a 1% TAE/agarose gel, 1 KB DNA ladder (Zymo Research).

Reproducible Sample Processing



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

Protocol

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

(I) Buffer Preparation

- ✓ Add 20 ml (R2135) or 80 ml (R2136) isopropanol to the **MagBead DNA/RNA Wash 1** concentrate.
- ✓ Add 30 ml (R2135) or 120 ml (R2136) isopropanol to the **MagBead DNA/RNA Wash 2** concentrate.
- ✓ Reconstitute lyophilized **Proteinase K** at 20 mg/ml with **Proteinase K Storage Buffer** and mix by vortexing. Use immediately or store frozen aliquots:
  #D3001-2-20 (20 mg), add 1.2 ml **buffer**
- ✓ Reconstitute each vial of lyophilized **DNase I** with **DNase/RNase-Free Water** in a conical tube (not provided). Mix by gentle inversion and store frozen aliquots.
  #E1011-A (1500 U), add 13.5 ml **water**
  #E1009-A (250 U), add 2.25 ml **water**

  For each sample to be treated, prepare **DNase I Reaction Mix** (scale up proportionally): Add 45 µl **DNase I** (reconstituted) and 5 µl **DNA Digestion Buffer** in a nuclease-free tube (not provided), mix by gentle inversion and place on ice until ready to use.

(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) and mix. If a sample is already collected in **DNA/RNA Shield™**, proceed to step 2.

Sample Type	Maximum Input
Soil, feces, plant, seed	≤ 50 mg
Cells in DNA/RNA Shield™ or isotonic buffer/PBS (bacterial $2x10^8$, yeast $2x10^7$, mammalian $2x10^6$)	≤ 5-20 mg (wet weight)
DNA/RNA Shield™ collection devices (e.g., cat. #R1101, R1102-R1105) or	750 µl
Biological liquids and swab collections (e.g., cat. #R1100, R1106-R1109, R1150)	750 µl

2. For complete microbial lysis, perform mechanical homogenization via bead beating (recommended: ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm, S6012-50) or ZymoBIOMICS BashingBead™ Lysis Rack (0.1 & 0.5 mm, S6002-96-7); available separately).

   When using the ZymoBIOMICS BashingBead™ Lysis Rack (0.1 & 0.5 mm, S6002-96-7), it is recommended to seal the rack with the provided sealing foils. Seal the foils to the rack using a heat sealing device set at 180°C for 4 seconds. Refer to Appendix C for more detailed guidance.

3. Transfer entire liquid sample into a lysis tube/rack and secure in a high-speed bead beater (e.g., MP Bio FastPrep, Bertin Precellys, etc.). Process[1] at maximum speed for ≥ 5 minutes.
4. Centrifuge and transfer up to 200 µl of the supernatant[2] into a nuclease-free tube (not provided).
5. Add 10 µl **Proteinase K** for every 200 µl sample**.** Mix and incubate at room temperature (20-30ºC) for 30 minutes.
6. Proceed to purification, page 6 (Total Nucleic Acid) or page 7 (DNA and RNA).

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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 200 µl sample can be processed per prep.

(III) Total Nucleic Acid Purification

1. Add 200 µl (1 volume) **DNA/RNA Lysis Buffer** to 200 µl sample and mix well[1].
2. Add 400 µl ethanol (95-100%) to the sample and mix well[1].
3. Add 30 µl **ZymoBIOMICS™ MagBinding Beads** and mix well[1] 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 stand[2] until beads have pelleted, then aspirate[3] and discard the cleared supernatant.
5. Add 500 µl **MagBead DNA/RNA Wash 1** and mix well[1]. Pellet the beads[2,3] and discard the supernatant.
6. Add 500 µl **MagBead DNA/RNA Wash 2** and mix well[1]. Pellet the beads[2,3] and discard the supernatant.
7. Add 500 µl ethanol (95-100%) and mix well[1]. 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 well[1] 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 dry[4].
11. To elute DNA/RNA from the beads, add 50 µl **ZymoBIOMICS™ DNase/RNase-Free Water** and mix well[1] for 5 minutes.
12. Transfer the plate/tube to the magnetic stand[2] until beads have pelleted, then aspirate[3] and dispense the eluted DNA/RNA to a new plate/tube.

    The eluted DNA/RNA can be used immediately or stored frozen.

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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 (25-55ºC).

(IV) DNA and RNA Purification (in two separate fractions)

1. Add 500 µl (2.5 volumes) **DNA/RNA Lysis Buffer** to the 200 µl sample and mix well[1].
2. Add 30 µl **MagBinding Beads** and mix well for 20 minutes.

   Important: **ZymoBIOMICS™ MagBinding Beads** settle quickly, ensure that beads are kept in suspension while dispensing.
3. Transfer the plate/tube to the magnetic stand[2] until beads (DNA) have pelleted, then transfer[3] the cleared supernatant (RNA) into a new plate/tube.

DNA Purification (beads)	**RNA Purification** (supernatant)
4. Add 500 µl **MagBead DNA/RNA Wash 1** and mix well[1]. Pellet the beads[2,3] and discard the supernatant.	4. Add 700 µl (1 volume) ethanol (95-100%) to the supernatant and mix well[1].
5. Add 500 µl **MagBead DNA/RNA Wash 2** and mix well[1]. Pellet the beads[2,3] and discard the supernatant.	5. Add 30 µl/well **MagBinding Beads** and mix well[1] for 10 minutes.
6. Add 500 µl ethanol (95-100%) and mix well[1]. Pellet the beads[2,3] and discard the supernatant.	6. Transfer the plate/tube to the magnetic stand[2] until beads have pelleted, then aspirate[3] and discard the cleared supernatant.
7. Repeat step 6.	7. Add 500 µl **MagBead DNA/RNA Wash 1** and mix well[1]. Pellet the beads[2,3] and discard the supernatant.
8. Dry the beads for 10 minutes or until dry[4].	8. Add 500 µl **MagBead DNA/RNA Wash 2** and mix well[1]. Pellet the beads[2,3] and discard the supernatant.

(Continue to DNA and RNA Purification, page 8.)

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 (25-55ºC).

DNA Purification (beads)

9. Add 50 µl **ZymoBIOMICS™ DNase/RNase-Free Water** and mix well[1] for 5 minutes.

10. Transfer the plate/tube to the magnetic stand[2] until beads have pelleted, then aspirate[3] and dispense the eluted DNA to a new plate/tube.

RNA Purification (supernatant)

9. Add 500 µl ethanol (95-100%) and mix well[1]. Pellet the beads[2,3] and discard the supernatant.

10. Repeat step 9.

11. **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 well[1] for 10 minutes. Pellet the beads[2,3] and discard the supernatant.
    - (D3) Repeat steps 9-10.

12. Dry the beads for 10 minutes or until dry[4]

13. Add 50 µl **ZymoBIOMICS™ DNase/RNase-Free Water** and mix well[1] for 5 minutes.

14. Transfer the plate/tube to the magnetic stand[2] until beads have pelleted, then aspirate[3] and dispense the eluted RNA to a new plate/tube.

The eluted DNA and RNA can be used immediately or stored frozen.

For footnotes, see page 7.

Appendices

Appendix A

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.

Appendix B

Automation Scripts

The **ZymoBIOMICS ™ MagBead RNA** (R2135/R2136) 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.

Appendix C

Heat Sealing ZymoBIOMICS™ BashingBead™ Lysis Racks

1. Remove all strip caps and use a Kimwipe to wipe away any residual beads found on the tops of the tubes.
2. Transfer samples into individual lysis rack tubes according to the sample input guide found in Step 1 of the main protocol (Page 5).
3. Cover the top of the lysis rack tubes with a Kimwipe and press down gently to remove any residual liquid.[1]
4. Place the lysis rack on the sealing device stage.
5. Center a sealing foil on top of the lysis rack, such that all tubes are covered by the foil with a slight overhang along each edge.
6. Set heat sealing device to 180°C and allow the device adequate time to reach the target temperature.
7. Press the sealer down onto the foil for 4 seconds to seal the rack.[2]

Note: *At no point should a roller (or similar) be used to press down the foil, as it can damage the bond between the sealing foil and lysis rack, causing leakage.*

8. Allow the sealed lysis rack to cool to room temperature before loading onto a mechanical lysis device.

Note: *Placing a rubber or silicone mat between the sealing foil and mounting clamp is recommended to protect the sealing foil from becoming worn or damaged by the clamp during mechanical lysis.*

9. Proceed with Step 3 of the main protocol (Page 5).

For best results, it is recommended to use a validated heat sealing device, such as the Vitl Variable Temperature Sealer (VTS) (V902001), found here: https://vitlproducts.com/products/variable-temperature-sealer.

1 Avoid leaving liquid on the tops of the tubes. Residual liquid may negatively affect subsequent heat sealing.
2 If different sealing foils or devices are used, heat sealing parameters may require user optimization.

Ordering Information

Product Description	Catalog No.	Size
ZymoBIOMICS™ MagBead DNA/RNA	R2135 R2136	96 preps. 4 x 96 preps.

Individual Kit Components	Catalog No.	Amount
DNA/RNA Shield™	R1100-50 R1100-250	50 ml 250 ml
DNA/RNA Lysis Buffer	D7001-1-50 D7001-1-200	50 ml 200 ml
DNA/RNA Prep Buffer	D7010-2-25 D7010-2-50	25 ml 50 ml
MagBead DNA/RNA Wash 1 (concentrate)	R2130-1-30 R2130-1-120	30 ml 120 ml
MagBead DNA/RNA Wash 2 (concentrate)	R2130-2-20 R2130-2-80	20 ml 80 ml
ZymoBIOMICS™ DNase/RNase-Free Water	D4302-5-30 D4302-5-50	30 ml 40 ml
DNase I (lyophilized) (Supplied with DNA Digestion Buffer, 4 ml)	E1010 E1011	1 set (250 U) 1 set (1500 U)
Proteinase K (lyophilized) **& Storage Buffer**	D3001-2-5 D3001-2-20	5 mg 20 mg
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	S6012-50	50 pack
ZymoBIOMICS BashingBead™ Lysis Rack (0.1 & 0.5 mm)	S6002-96-7	1x 96-well rack
ZymoBIOMICS™ MagBinding Beads	D4302-6-6 D4302-6-12	6 ml 12 ml

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 DNA/RNA	
Miniprep #R2002	Up to 250 mg soil, feces, microbes, water, etc.

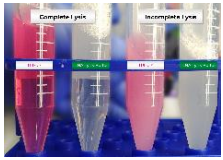
✓ For RNA clean-up (purification) from the aqueous phase (e.g., TRIzol, TRI Reagent or similar) or from any enzymatic reaction (e.g., DNase I treated RNA):

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)	Incomplete lysis and/or cellular debris: - Increase the volume of DNA/RNA Shield and/or RNA Lysis Buffer for complete lysis and homogenization. Be sure to centrifuge any cellular debris and then process the cleared lysate. Washing of 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.
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 nucleic acid recovery. Use less input material and/or increase the volume DNA/RNA Shield™ and/or RNA Lysis Buffer. High-protein content - Perform Proteinase K treatment to the sample prior to purification. See appropriate sample preparation protocol. 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 RNA to be sufficiently bound to beads.
DNA contamination	To remove DNA: - Perform DNase I treatment during purification (or post-purification, then re-purify the treated sample). - For future preps, increase the volume of DNA/RNA Shield™ and/or RNA Lysis Buffer to ensure complete lysis and homogenization of the sample.
RNA degradation	To prevent RNA degradation: - Immediately collect and lyse fresh sample into DNA/RNA Shield™ and/or RNA Lysis Buffer ensure stability. Homogenized samples 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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Zymo Research is committed to simplifying your research with quality products and services. If you are dissatisfied with this product for any reason, 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.

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