ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZymoBIOMICS™ MagBead DNA/RNA

Catalog Nos. **R2135, R2136**

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 DNA/RNA (including small/microRNAs) are inhibitor-free and ready for RT/qPCR and microbiome measurements using Next-Gen sequencing.
- **High-Sensitivity**: Increased detection limit of very low abundance organisms.

Contents

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.

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.

Product Contents

ZymoBIOMICS™ MagBead DNA/RNA (Kit Size)	**R2135** (96 Preps)	**R2136** (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.
DNA/RNA Lysis Buffer	2 x 50 ml	2 x 200 ml	Room Temp.
DNA/RNA Prep Buffer	2 x 50 ml	2 x 200 ml	Room Temp.
MagBead DNA/RNA Wash 1[1] (concentrate)	3 x 30 ml	3 x 120 ml	Room Temp.
MagBead DNA/RNA Wash 2[2] (concentrate)	3 x 20 ml	3 x 80 ml	Room Temp.
ZymoBIOMICS™ DNase/RNase-Free Water	30 ml	2 x 50 ml	Room Temp.
DNase I[3] (lyophilized)	3 x 250 U	2 x 1500 U	Room Temp. (-20°C; reconstituted)
DNA Digestion Buffer	4 ml	4 ml	Room Temp.
Proteinase K[3] (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 (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] Prior to use, reconstitute lyophilized **DNase I** and lyophilized **Proteinase K** according to page 3, Reagent Preparation.

Specifications

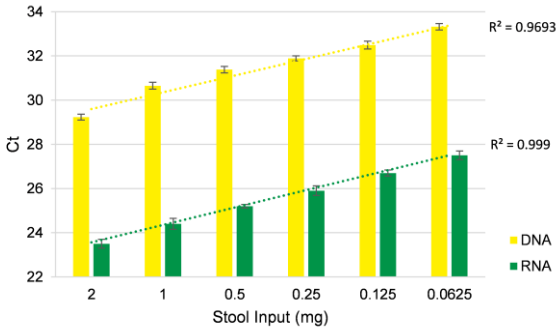
- **Sample Types** – Bacterial, fungal, protozoan, algae, viral, mitochondrial, and host DNA and RNA are efficiently isolated from ≤ 50 mg of mammalian feces, ≤ 50 mg soil, and 5-20 mg (wet weight) of fungal/bacterial cells, biofilms, water, and swabs.
- **Sample Preservation** – **DNA/RNA Shield™** lyses cells, inactivates nucleases and infectious agents and is ideal for safe sample storage and transport at ambient temperatures.
- **Size Limits** – Capable of recovering DNA and total RNA ≥17 nucleotides.
- **Purity** – High-quality RNA is ready for Next-Gen Sequencing, RT/PCR, hybridization, etc.
- **Binding Capacity** – 15 µg DNA/RNA per 30 µl **ZymoBIOMICS™ MagBinding Beads**.
- **Storage** – DNA and 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 or separator, heat block, liquid handler or robotic sample processer (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).

Product Description

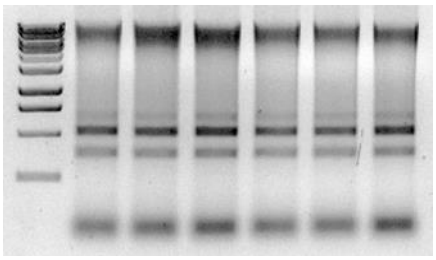
The **ZymoBIOMICS™ MagBead DNA/RNA** kit provides a high-throughput, magnetic bead-based purification of both high-quality DNA and total RNA (including small/microRNAs) from the same starting sample. The provided **DNA/RNA Shield™** inactivates infectious agents and is ideal for sample storage at ambient 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.*

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

High-Quality DNA & RNA

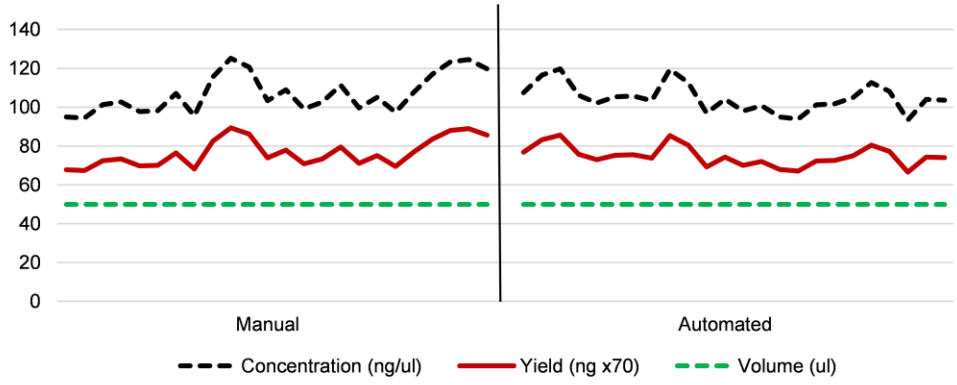


The **ZymoBIOMICS™ MagBead DNA/RNA** kit is able to purify DNA (top; yellow) and RNA (bottom; green) from human stool (at low biomass inputs). Results analyzed by qPCR (n=2).



DNA & RNA from human stool are high quality using the **ZymoBIOMICS™ MagBead DNA/RNA**. Elutions were analyzed in a 1% TAE/agarose/EtBr gel. The size marker is a 1 kb 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).

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.

Reagent 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.
- ✓ 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.
- ✓ Prepare **DNase I Reaction Mix** (according to the example below; scale as needed).

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 Total Nucleic Acid Purification, page 4 or RNA Purification (DNA & RNA Purification), page 5.

Protocol

The isolation consists of: (I) Sample Preparation, (II) Total Nucleic Acid Purification and/or (III) DNA and RNA Purification (in two separate fractions).

(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 (20-30ºC), unless specified.

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

Sample Type	Maximum Input
Feces	50 mg
Soil	50 mg
Liquid Samples[2] and Swab Collections	250 µl
Cells (Suspended 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 µl

2. To achieve unbiased lysis of different organisms, including hard-to-lyse microbes, perform mechanical homogenization[3] (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 (20-30ºC) for 30 minutes.

4. Proceed to Total Nucleic Acid Purification, page 4 or DNA and RNA Purification, page 5.

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™).

(II) 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.

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

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 (25-55ºC).

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).

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

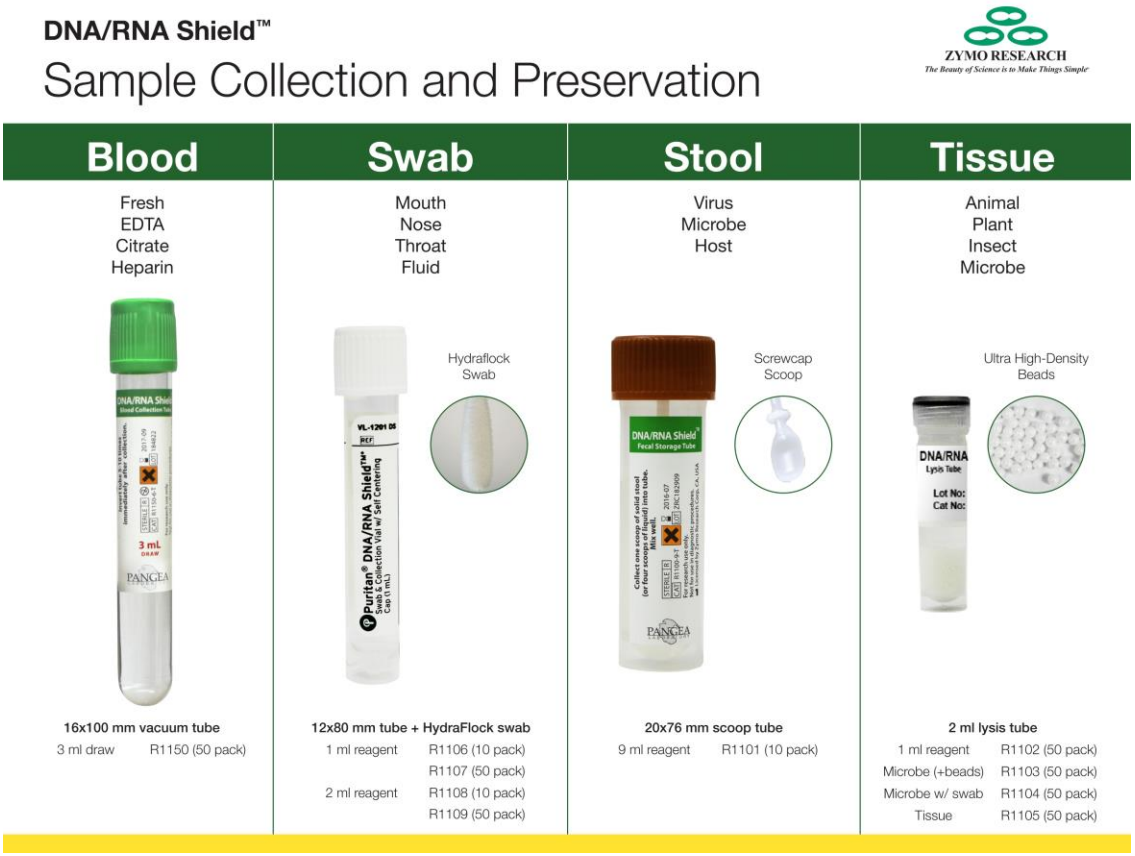
1. Add 500 µl (2.5 volumes) **DNA/RNA Lysis Buffer** to the 200 µl sample and mix well[1].
2. Add 30 µl **ZymoBIOMICS™ MagBinding Beads** and mix well for 20 minutes.

   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 **ZymoBIOMICS™ 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.
9. Add 50 µl **ZymoBIOMICS™ DNase/RNase-Free Water** and mix well[1] for 5 minutes.	9. Add 500 µl ethanol (95-100%) and mix well[1]. Pellet the beads[2,3] and discard the supernatant.
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.	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 & RNA can be used immediately or stored frozen.

Appendix A: Compatible DNA/RNA Shield™ Collection Devices



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

Automation Scripts

The **ZymoBIOMICS™ MagBead DNA/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.

Troubleshooting

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

Problem	Possible Causes and Suggested Solutions
Low Recovery	
Binding Conditions	• **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 scale up volume of DNA/RNA Lysis Buffer, magnetic beads and elution volume proportionally.
Elution Parameters	• **Elution Volume:** Elution volume can be increased to ensure adequate recovery (e.g., ≥50 µl). • **Adjust Temperature:** Increase elution temperature to 55ºC. This can improve recovery if beads are "sticky" (for high molecular weight genomic DNA, etc.).
Low Purity ($A_{260/230\ nm}$)	
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.
Drying of Beads	• **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	
Sample Input	• **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.
Bead-beating	• **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 on bead-beating, read Zymo Research's article, "The Lysis Bias Crisis".

Ordering Information

Product Description	Catalog No.	Kit Size
ZymoBIOMICS™ MagBead DNA/RNA	R2135 R2136	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
DNA/RNA Lysis Buffer	D7001-1-50 D7001-1-200	50 ml 200 ml
DNA/RNA Prep Buffer	D7010-2-50 D7010-2-200	50 ml 200 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


ZYMO RESEARCH
The Beauty of Science is to Make Things Simple