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ZymoBIOMICS MagBead DNA/RNA-R2135

ZYMO Research Corp.[1]

[1]www.zymoresearch.com

1 Works for me dx.doi.org/10.17504/protocols.io.bktxkwpn

XENOGENE

Chris Costa
XENOGENE

ABSTRACT

The ZymoBIOMICSTM 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 ShieldTM 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 ZymoBIOMICSTM DNase/RNase-Free Water. DNA/RNA is ready for any downstream application including Next-Gen Sequencing, RT-qPCR, etc.

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.

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GUIDELINES

- **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 ShieldTM 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)

MATERIALS TEXT

- 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)

SAFETY WARNINGS

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.

DISCLAIMER:

BEFORE STARTING

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

Reconstitute DNase I with ZymoBIOMICSTM 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.
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.

Sample Preparation 30m

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- 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.

750 µl DNA/RNA Shield Add to a sample 250 uL max.input and mix and/or homogenize.

Sample Type	Maximum Input
Feces	50 mg
Soil	50 mg
Liquid Samples and Swab Collections	250 ul
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 ul

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 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 (20-30oC) for 30 minutes.

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

Total Nucleic Acid Purification 1h

5 **200 µl DNA/RNA Lysis Buffer** Add to 200 ul sample and mix well.

6 **400 µl ethanol (95-100%)** Add to the sample and mix well.

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30 µl ZymoBIOMICS MagBinding Beads Add and mix well for 20 minutes.

Important: ZymoBIOMICS MagBinding Beads settle quickly, ensure that beads are kept in suspension while dispensing.

8 Transfer the plate/tube to the magnetic stand until beads have pelleted, then aspirate and discard the cleared supernatant.

9 **500 µl MagBead DNA/RNA Wash 1** Add and mix well. Pellet the beads discard the supernatant.

10 **500 µl MagBead DNA/RNA Wash 2** Add and mix well. Pellet the beads discard the supernatant.

11 **500 µl ethanol (95-100%)** Add and mix well. Pellet the beads discard the supernatant.

12 **go to step #11** Repeat once.

13 DNase I treatment (optional)

13.1 Add 50 µl DNase I Reaction Mix and mix gently for 10 minutes.

13.2 Add 500 µl DNA/RNA Prep Buffer and mix well1 for 10 minutes. Pellet the beads2,3 and discard the supernatant.

13.3 **go to step #11** Repeat once.

14 Dry the beads for 10 minutes or until dry.

15 To elute DNA/RNA from the beads, add 50 µl ZymoBIOMICS DNase/RNase- Free Water and mix well1 for 5 minutes.

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16 Transfer the plate/tube to the magnetic stand until beads have pelleted, then aspirate and dispense the eluted DNA/RNA to a new plate/tube.

DNA and RNA Purification 40m

17 **500 µl DNA/RNA Lysis Buffer** Add to the 200 ul sample and mix well.

18 

30 µl ZymoBIOMICS MagBinding Beads Add and mix well for 20 minutes.

Important: ZymoBIOMICS MagBinding Beads settle quickly, ensure that beads are kept in suspension while dispensing.

19 Transfer the plate/tube to the magnetic stand until beads (DNA) have pelleted, then transfer the cleared supernatant (RNA) into a new plate/tube.

Step 19 includes a Step case.
DNA
RNA

step case

DNA

20 **500 µl MagBead DNA/RNA Wash 1** Add and mix well. Pellet the beads and discard the supermatant.

21 **500 µl MagBead DNA/RNA Wash 2** Add and mix well. Pellet the beads and discard the supermatant.

22 **500 µl ethanol (95-100%)** Add and mix well. Pellet the beads and discard the supermatant.

23 **go to step #21** Repeat once.

24 Dry the beads for 10 minutes or until dry.

25 **50 µl ZymoBIOMICS DNase/RNase-Free Water** Add and mix well for 5 minutes.

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26 Transfer the plate/tube to the magnetic stand until beads have pelleted, then aspirate and dispense the eluted DNA to a new plate/tube.

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