ZYMO RESEARCH

RNA Purification Made Simple™

Quick-RNA™ Fecal/Soil Microbe Microprep

RNA from any tough-to-lyse sample

Highlights

- Quick, spin-column purification of total RNA (including small/microRNAs) from bacteria, fungi, protozoa, protist, algae, viral, host, etc. from various soil (sludge, sediment, etc.) and fecal samples (mammalian, avian, etc.).
- ZR BashingBead™ Lysis Tubes are ultra-high density, fracture resistant, chemically inert ceramic beads and used for the robust homogenization of any tough-to-lyse sample.
- RNA is ready for Next-Gen Sequencing, RT/qPCR and any downstream application, etc.

Catalog Numbers:
R2040

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

Quick-RNA™ Fecal/Soil Microbe Microprep	R2040 (50 prep)
S/F RNA Lysis Buffer	50 ml
RNA Binding Buffer	50 ml
RNA Prep Buffer	25 ml (x2)
RNA Wash Buffer[1] (concentrate)	24 ml
DNase/RNase-Free Water	6 ml
Prep Solution	30 ml
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	50
Zymo-Spin™ IIICG Columns	50 (x2)
Zymo-Spin™ IC Columns	50
Zymo-Spin™ III-HRC Filters	50
Collection Tubes	200
Instruction Manual	1

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature.
Before use:
1 Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.

Specifications

- **Sample Sources –** Up to 250 mg of soil (sludge, sediment, etc.) and/or fecal samples (mammalian, avian, etc.) that contain tough-to-lyse bacteria, fungi, protozoa, protist, algae, viruses including host.
- **Size –** Total RNA including small/microRNAs (≥ 17 nt).
- **Purity –** A_{260}/A_{280} & A_{260}/A_{230} > 1.8. RNA is ready for Next-Gen Sequencing, RT/qPCR, etc.
- **Binding Capacity – Zymo-Spin™ IC Column** yield up to 10 µg RNA.
- **Elution Volume –** ≥ 6 µl **DNase/RNase-Free Water.**
- **Equipment Needed (user provided) –** Microcentrifuge, vortex, and a high-speed homogenizer/cell disruptor or bead beater (e.g., MP Bio FastPrep-24, Bertin Precellys, etc.) (recommended).

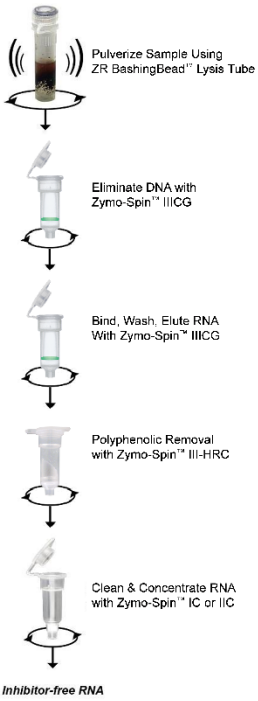
Product Description

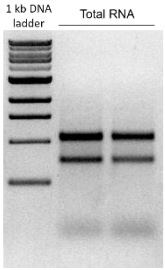
The **Quick-RNA™ Fecal/Soil Microbe Microprep** kit is an innovative product designed for rapid isolation of total RNA including small RNAs (>17 nt) from up to 250 mg of soil (sludge, sediment, etc.) and/or fecal samples (mammalian, avian, etc.) that contain tough-to-lyse bacteria, fungi, protozoa, protist, algae, viruses including host.

The kit includes unique technology such as the **ZR BashingBead™ Lysis Tubes** and features a specially formulated **S/FRNA Lysis Buffer**. The **Zymo-Spin™ IIICG Column** allows for high-capacity nucleic acid binding and the subsequent **Zymo-Spin™ IC Column** efficiently adsorbs and concentrates total RNA.

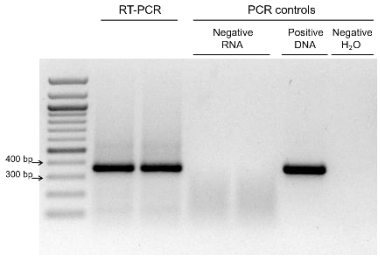
The RNA is washed and then eluted with **DNase/RNase-Free Water**. For inhibitor removal, the eluted RNA can be treated by running the sample through the **Zymo-Spin™ III-HRC Filters**. RNA is eluted in as little as 6 µl and is suitable for subsequent procedures including RT-qPCR.

Efficient Recovery of RNA from Any Soil or Fecal Sample





Total RNA isolation of Arthrobacter sp. from 250 mg sludge using the Quick-RNA™ Fecal/Soil Microbe Microprep kit in duplicate. ZR 1 kb DNA ladder, Zymo Research, #M5006-50.



PCR amplification of Arthrobacter sp. rRNA transcript (361 bp fragment shown) in duplicate: ZR 100 bp DNA ladder, Zymo Research, #M5005-50. PCR controls: Negative control - Total RNA isolation from Arthrobacter sp. in 250 mg sludge in duplicate (above). Positive control - Arthrobacter sp. genomic DNA. Negative control - water.

Protocol

The protocol consists of: (I) Buffer Preparation and (II) Total RNA Purification

(I) Buffer Preparation

- ✓ Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer**.

(II) Total RNA Purification

✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

1. Transfer up to 250 mg soil and/or fecal sample into a **ZR BashingBead Lysis Tube** (0.1 & 0.5 mm) and add 1 ml **S/F RNA Lysis Buffer**.
2. Secure tube in a bead beater fitted with a 2 ml tube hold assembly and process. See example below:

Homogenizers	Bead-Beating Time
High-speed (e.g., MP Bio FastPrep-24, Bertin Precellys)	30 – 60 sec
Low-speed (e.g., Disruptor Genie)	5 – 10 min

3. Centrifuge the tube for 1 minute to pellet debris.
4. Transfer the cleared supernatant into a nuclease-free tube (not provided) and add 1 volume of **RNA Binding Buffer** (1:1). Mix well.

   Example: Add 400 µl **RNA Binding Buffer** to 400 µl cleared supernatant.
5. Transfer the mixture (step 4) in to a **Zymo-Spin™ IIICG Column**[1] in a **Collection Tube** and centrifuge at 3,000 x g for 30 seconds. Save the flow-through!
6. To the flow-through, add an equal volume ethanol (95-100%) (1:1) and mix well.

   Example: Add 800 µl ethanol to 800 µl flow-through.
7. Transfer the mixture into a new **Zymo-Spin™ IIICG Column**[2] in a **Collection Tube** and centrifuge. Discard the flow-through.
8. Add 400 µl **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through and transfer the column into a nuclease-free tube (not provided).
9. Add 100 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge. Set the eluate aside.
10. Place a **Zymo-Spin™ III-HRC Filter** in a new **Collection Tube** and add 600 µl **Prep Solution**. Centrifuge at 8,000 x g for 3 minutes.
11. Transfer the eluted RNA (step 9) into the prepared filter in a nuclease-free tube (not provided) and centrifuge at 16,000 x g for 3 minutes.

(Continue to page 6 for Total RNA Purification.)

1 To process samples > 700 µl, columns may be reloaded.

12. Add 200 µl **RNA Binding Buffer** to the filtered RNA (step 11) (2:1) and mix well.

13. Add an equal volume ethanol (95-100%) (1:1) and mix well.

14. Transfer the mixture into a new **Zymo-Spin™ IC Column**[1] in a **Collection Tube** and centrifuge. Discard the flow-through.

    Optional: At this point, DNase I treatment can be performed. See page 8.

15. Add 400 µl **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.

16. Add 700 µl **RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.

17. Add 400 µl **RNA Wash Buffer** and centrifuge the column for 1 minute to ensure complete removal of the wash buffer. Then carefully, transfer the column into a nuclease-free tube (not provided).

18. Add 15 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

    Alternatively, for highly concentrated RNA use ≥ 6 µl elution.

    The eluted RNA can be used immediately or stored frozen.

1 To process samples > 700 µl, columns may be reloaded.

Appendices

Samples stabilized and stored in DNA/RNA Shield™

✓ If frozen, thaw sample in **DNA/RNA Shield™** to room temperature (20-30°C). Mix well by vortex.

Homogenized Sample

1. Transfer 400 µl of sample homogenized in **DNA/RNA Shield™** to a new RNase-free tube (not provided).

2. Add an equal volume of **S/F RNA Lysis Buffer** (1:1) and mix well.

3. Proceed to Total RNA Purification (page 5, step 3).

Non-homogenized Sample

1. Transfer 800 µl - 1 ml of sample suspended in **DNA/RNA Shield™** to a **ZR BashingBead Lysis Tube**.
2. Secure the **ZR BashingBead Lysis Tube** in a bead beater fitted with a 2 ml tube holder assembly and process (see homogenization suggestions on page 5, step 2).
3. Centrifuge the **ZR BashingBead Lysis Tube** for 1 minute at high speed (e.g., 16,000 x g).
4. Transfer 400 µl of the supernatant to a new RNase-free tube (not provided).
5. Add 400 µl of **S/F RNA Lysis Buffer** (1:1) to the supernatant and mix well.
6. Proceed to Total RNA Purification (page 5, step 4).

(Appendices continued)

DNase I Treatment (in-column)

- ✓ Perform DNase I treatment with DNase I Set (#E1010) and RNA Wash Buffer (concentrate; #R1003-3-6); available separately.
- ✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

1. Following RNA binding step (page 5, step 6), add 400 µl **RNA Wash Buffer** to the column, centrifuge and discard the flow-through.
2. For each sample to be treated, prepare **DNase I Reaction Mix** (see table below) in an RNase-free tube (not provided) and mix by gentle inversion. Then add 40 µl directly into column matrix and incubate at room temperature (20-30°C) for 15 minutes. Proceed with the purification (page 6, step 15).

DNase I Reaction Mix	
DNase I (reconstituted; 1 U/µl)[1]	5 µl
DNA Digestion Buffer	35 µl

1 Prior to use, reconstitute the lyophilized **DNase I** with 275 µl DNase/RNase-Free Water. Mix by gentle inversion and store frozen aliquots. * Unit definition – one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A_{260} units/ml of reaction mixture at 25°C.

Ordering Information

Product Description	Catalog No.	Size
Quick-RNA™ Fecal/Soil Microbe Microprep	R2040	50 preps.

Individual Kit Components	Catalog No.	Amount
RNA Lysis Buffer	R1060-1-50 R1060-1-100	50 ml 100 ml
RNA Prep Buffer	R1060-2-25 R1060-2-100	25 ml 100 ml
RNA Wash Buffer (concentrate)	R1003-3-24 R1003-3-48	24 ml 48 ml
DNase/RNase-Free Water	W1001-10 W1001-30	10 ml 30 ml
DNase I Set (lyophilized) (250 U supplied with DNA Digestion Buffer, 4 ml)	E1010	1 set
Zymo-Spin™ IIICG Columns	C1006-50-G	50
Zymo-Spin™ IC Columns	C1004-50	50
Zymo-Spin™ III-HRC Filters	C1058-50	50
Collection Tubes	C1001-50	50
OneStep PCR Inhibitor Removal Kit	D6030	50
Prep Solution	D6035-1-30	30 ml
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	S6012-50	50

Complete Your Workflow

✓ For tough-to-lyse samples, use ZR BashingBead Lysis Tubes:

ZR BashingBead Lysis Tubes	
2.0 mm beads #S6003	Plant/animal tissue
0.1 + 0.5 mm beads #S6012	Microbes
0.1 + 2.0 mm beads #S6014	Microbes in tissue/insects

✓ For isolation of RNA from any sample:

Quick-RNA kits	
Miniprep Plus #R1057/R1058	≤ 10^7 cells, ≤ 50 mg tissue
MagBeads #R2132/R2133	Automatable (Tecan, Hamilton, Kingfisher, etc.)

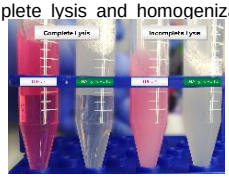
✓ For clean-up (purification) and concentration of any RNA sample. (e.g., from the aqueous phase of TRIzol® extractions) or from any enzymatic reaction (e.g., DNase I treated RNA):

RNA Clean & Concentrator kits	
Microprep #R1013-R1014	DNase I Set included
MagBeads #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)	Sample handling: - Ethanol and/or salt contamination. After centrifugation steps, carefully remove the column from the collection tube to prevent buffer carryover. Alternatively, blot emptied collection tube with a tissue or towel. - Make sure lysate and wash buffers have passed completely through the matrix of the column. This may require centrifuging at a higher speed and/or longer time. Incomplete lysis and/or cellular debris: - Increase the volume DNA/RNA Shield™ and/or RNA Lysis Buffer (proportionally) to ensure complete lysis and homogenization. Be sure to centrifuge any cellular debris and then process the cleared lysate.
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.
DNA contamination	To remove DNA: - Perform in-tube DNase I treatment post-purification, refer to the RNA Clean & Concentrator (Cat. R1013) protocol, page 6, "DNase I treatment before RNA clean-up". Then, add 150 µl RNA Lysis Buffer to the 50 µl reaction mix (3:1) and mix well. Add an equal volume ethanol (95-100%) (1:1) and mix well. Proceed to purification step 7, page 5. - In the future, Perform in-column DNase I treatment, step 7, page 5. - 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

100% satisfaction guarantee on all Zymo Research products, or your money back.

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.

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.

ZYMO RESEARCH

The **BEAUTY** *of* **SCIENCE** *is to Make Things* **SIMPLE**®

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