



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.



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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 μ g RNA.
- **Elution Volume** – ≥ 6 μ l 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).

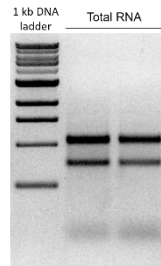
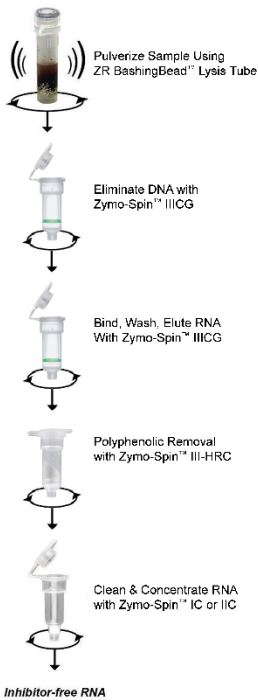
Product Description

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.

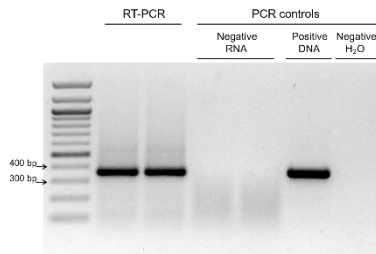
The kit includes unique technology such as the **ZR BashingBead™ Lysis Tubes** and features a specially formulated **S/FRNA Lysis Buffer**. The **Zymo-Spin™ IICG Column** allows for high-capacity nucleic acid binding and the subsequent **Zymo-Spin™ IC Column** efficiently adsorbs and concentrates total 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., Vortex 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 Column¹** 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 Column²** 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.

(Total RNA Purification continued)

12. Add 200 μ l **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 μ l **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
16. Add 700 μ l **RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.
17. Add 400 μ l **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 μ l **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

Alternatively, for highly concentrated RNA use ≥ 6 μ l elution.

The eluted RNA can be used immediately or stored frozen.

¹ To process samples > 700 μ l, 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 6, step 14), 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 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 A₂₆₀ 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™ IICG 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.)

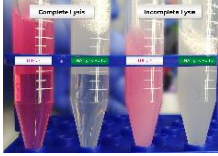
- ✓ For clean-up (purification) and concentration of any RNA sample. (e.g., from the aqueous phase of TRIzol[®] extractions) or from any enzymatic reaction (e.g., DNase I treated RNA):

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	<p>Incomplete lysis and/or high-mass input:</p> <ul style="list-style-type: none"> - 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)	<p>Sample handling:</p> <ul style="list-style-type: none"> - 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. <p>Incomplete lysis and/or cellular debris:</p> <ul style="list-style-type: none"> - 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	<p>Sample input:</p> <ul style="list-style-type: none"> - 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. <p>High-protein content:</p> <ul style="list-style-type: none"> - Perform Proteinase K treatment to the sample prior to purification. See appropriate sample preparation protocol.
DNA contamination	<p>To remove DNA:</p> <ul style="list-style-type: none"> - 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	<p>To prevent RNA degradation:</p> <ul style="list-style-type: none"> - 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



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