ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Quick-RNA™ Fecal/Soil Microbe Microprep Kit

Catalog No. **R2040**

Highlights

- Quick, 10 minute isolation of total RNA (~10 µg) from various soil and fecal samples using ultra-high density *BashingBeads*™ and *Zymo-Spin*™ column technologies.
- High-quality RNA eluted in ≥6 µl is ready for reverse transcription, microarray, sequencing, etc.

Contents

ZYMO RESEARCH CORP.

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Satisfaction of all Zymo Research products is guaranteed. If you are dissatisfied with this product please call 1-888-882-9682.

For assistance, contact us at tech@zymoresearch.com.

Product Contents

***Quick*-RNA™ Fecal/Soil Microbe Microprep Kit** (Kit Size)	**R2040** (50 Preps.)	**Storage Temperature**
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	50	Room Temp.
S/F RNA Lysis Buffer	50 ml	Room Temp.
RNA Binding Buffer	50 ml	Room Temp.
RNA Prep Buffer	2x 25 ml	Room Temp.
RNA Wash Buffer[1] (concentrate)	24 ml	Room Temp.
DNase/RNase-Free Water	6 ml	Room Temp.
Prep Solution	30 ml	Room Temp.
Zymo-Spin™ IC Columns	50	Room Temp.
Zymo-Spin™ IIICG Columns	2x 50	Room Temp.
Zymo-Spin™ III-HRC Filters	50	Room Temp.
Collection Tubes	4x 50	Room Temp.
Instruction Manual	1	-

Note - 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.

[1] Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate before use.

Specifications

- **Sample Types** – Bacteria, fungi, protozoa, and algae in soil, sludge or sediments, and bacteria, protist and/or host RNA from feces (mammalian, avian, etc.).
- **Sample Size** – ≤250 mg
- **Format** – Bead beating, spin column.
- **RNA Purity** – High quality RNA (A_{260}/A_{280} >1.8, A_{260}/A_{230} >1.8) suitable for all downstream RNA-based manipulations.
- **Yield** – Up to 10 µg RNA can be eluted into ≥6 µl RNase-free water allowing for a highly concentrated sample.
- **RNA Storage** – RNA is eluted with RNase-free water and can be stored at ≤-70 ºC. The addition of RNase inhibitors is highly recommended for prolonged storage.
- **Required Equipment** – Microcentrifuge, vortex, cell disrupter/pulverizer (optional).

Notes:

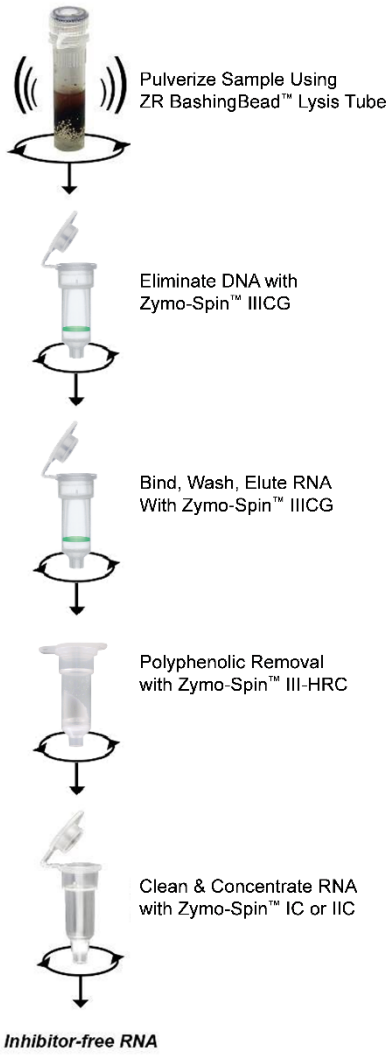
This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

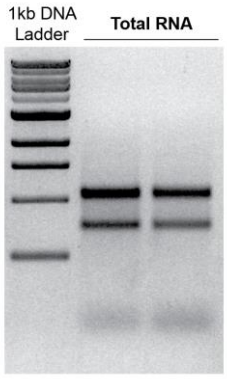
Product Description

The ***Quick*-RNA™ Fecal/Soil Microbe Microprep Kit** is an innovative product designed for the simple, reliable, and rapid isolation of total RNA including small RNAs (>17 nt) from various soil, sludge, sediment and/or fecal samples. The procedure successfully isolates RNA from *tough-to-lyse* bacteria, fungi, protozoa (protist), algae, *etc.* in soil, and host RNA from fecal samples.

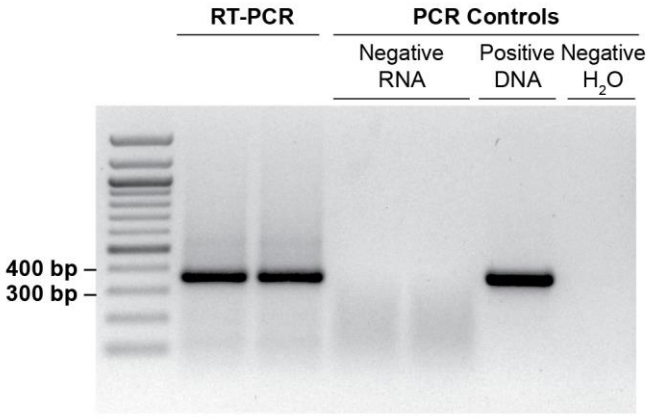
Samples are added to the **ZR BashingBead™ Lysis Tube** with an optimally designed **S/F RNA Lysis Buffer** where microbes are then lysed by bead beating to extract total RNA. The Zymo-Spin™ column technology allows for quick filtration, genomic DNA removal from sample lysates, and isolation of the RNA. **Zymo-Spin™ III-HRC Filter** separates RT-PCR inhibitors *(e.g., humic acids, polyphenols, tannins)* and the total RNA is concentrated using the **Zymo-Spin™ IC Column** with a minimum elution volume of ≥6 µl.

The result is highly-concentrated, purified RNA that is a suitable for subsequent RNA-based methods including RT-PCR, hybridization, *etc*.





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, Cat. No. 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.

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

Make sure guidelines are followed to ensure the RNA isolation procedure is performed in an RNase-free environment.

Notes:

[1] Up to 250 mg soil or feces can be processed.

[2] Processing times may be as little as 30 seconds when using high-speed (force) cell disruptors *(e.g.,* FastPrep®-24, or similar*)*. See manufacturer's literature for operating information.

[3] Sample *(i.e., supernatant)* and reagent volumes in this protocol can be adjusted proportionally if needed.

[4] To process samples >800 µl, reload the column.

[5] At this point, RNA samples can be in-column DNase I treated (page 5).

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

Reagent Preparation

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

Protocol

All centrifugation steps should be performed at 10,000-16,000 *x g*, unless specified otherwise.

1. Collect sample[1] into a **ZR BashingBead™ Lysis Tube** and add 1 ml **S/F RNA Lysis Buffer**.
2. Secure in a bead beater fitted with a 2 ml tube holder assembly and process[2].
3. Centrifuge the **ZR BashingBead™ Lysis Tube** for 1 minute.
4. Transfer 400 µl of the supernatant[3] into an RNase-free tube (not provided) and add 1 volume of **RNA Binding Buffer** to the supernatant. Mix well.
5. Transfer the mixture (step 4) into a **Zymo-Spin™ IIICG Column**[4] in a **Collection Tube** and centrifuge at ≥3,000 x *g* for 30 seconds. Save the flow-through!
6. Add 1 volume ethanol (95-100%) to the flow-through (step 5) in the **Collection Tube** and mix well.
7. Transfer the mixture (step 6) into a new **Zymo-Spin™ IIICG Column**[4] in a **Collection Tube** and centrifuge for 30 seconds. Discard the flow-through.
8. Add 400 µl **RNA Prep Buffer** to the column and centrifuge for 30 seconds. Then transfer the column into an RNase-free tube (not provided).
9. Add 100 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge for 30 seconds.
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 a prepared Zymo-Spin™ III-HRC Filter in an RNase-free tube (not provided) and centrifuge at exactly 16,000 x *g* for 3 minutes.
12. Add 200 µl **RNA Binding Buffer** to the filtrate and mix well
13. Add 300 µl ethanol (95-100%) and mix well.
14. Transfer the mixture (step 13) into a **Zymo-Spin™ IC Column**[4] in a **Collection Tube** and centrifuge for 30 seconds[5]. Discard the flow-through.
15. Add 400 µl **RNA Prep Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through
16. Add 700 µl **RNA Wash Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
17. Add 400 µl **RNA Wash Buffer** to the column and centrifuge for 2 minutes to ensure complete removal of the wash buffer. Transfer the column carefully into an RNase-free tube (not provided).
18. Add 15 µl of **DNase/RNase-Free Water**[6] directly to the column matrix and centrifuge for 30 seconds. The eluted RNA can be used immediately or stored at -70°C.

Appendix A: *In-Column* DNase I Digestion

The DNase I digestion procedure can be performed using **DNase I Set** (E1010)[1].
All centrifugation steps should be performed at 10,000-16,000 *x g.*

1. Following the RNA binding step (page 3, step 13), prewash the column with 400 µl **RNA Wash Buffer**. Centrifuge for 30 seconds. Discard the flow-through.

2. For each sample to be treated, prepare **DNase I Reaction Mix** in an RNase-free tube (not provided). Mix well by gentle inversion:

DNase I	5 µl
DNA Digestion Buffer	35 µl

3. Add 40 µl of the **DNase I Reaction Mix** directly to the column matrix. Incubate the column at room temperature (20-30°C) for 15 minutes. Then continue with RNA Purification (page 3, step 14).

Notes:

[1] Prior to use, reconstitute the lyophilized **DNase I** as indicated on the vial. 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/min/ml of reaction mixture at 25°C.

Ordering Information

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

For Individual Sale	Amount	Catalog No.
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	50	S6012-50
S/F RNA Lysis Buffer	50 ml	R2040-1-50
RNA Binding Buffer	25 ml 50 ml 100 ml 1000 ml	R1013-2-25 R1013-2-50 R1013-2-100 R1013-2-1000
RNA Prep Buffer	10 ml 25 ml	R1060-2-10 R1060-2-25
RNA Wash Buffer (concentrate)	6 ml 12 ml 24 ml 48 ml	R1003-3-6 R1003-3-12 R1003-3-24 R1003-3-48
DNase/RNase-Free Water	1 ml 4 ml 6 ml 10 ml	W1001-1 W1001-4 W1001-6 W1001-10
Zymo-Spin™ IC Columns	50 250	C1004-50 C1004-250
Zymo-Spin™ IIICG Columns	50 250	C1006-50-G C1006-250-G
OneStep™ PCR Inhibitor Removal Kit	50	D6030
Collection Tubes	50 500 1000	C1001-50 C1001-500 C1001-1000

Description	Amount	Cat. No.
Disruptor Genie™, 120V w/ 2 ml tube holder assembly.	1 unit	S6001-2-120
Disruptor Genie™, 240V w/ 2 ml tube holder assembly.	1 unit	S6001-2-240
TurboMix Attachment, 2 ml Permanently mounts to most existing Vortex Genie™ mixers converting them to a Disruptor Genie™.	1 unit	S6004-2

The **Disruptor Genie™** with 2 ml tube holder from Scientific Industries, Inc. (Cat. No. S6001-2 - Zymo Research Corp.)

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