ZYMO RESEARCH

RNA Purification Made Simple™

Quick-RNA™ Fungal/Bacterial Miniprep

RNA from any tough-to-lyse sample

Highlights

- Quick, spin-column purification of total RNA (including small/microRNAs) from any tough-to-lyse gram-positive (or negative) bacteria or fungi samples.
- 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:
R2014

Scan with your smart-phone camera to view the online protocol/video.

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Product Contents

Quick-RNA™ Fungal/Bacterial Miniprep	R2014 (50 prep)
RNA Lysis Buffer	50 ml
RNA Prep Buffer	25 ml
RNA Wash Buffer[1] (concentrate)	24 ml
DNase/RNase-Free Water	4 ml
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	50
Zymo-Spin™ IIICG Columns	50
Zymo-Spin™ IICR Columns	50
Collection Tubes	100
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 50-100 mg (wet weight) bacteria or fungi. This equates to approximately 10^9 bacterial cells or 10^8 yeast cells.
- **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™ IICR Column** yield up to 50 µg RNA.
- **Compatibility –** For samples stored in **DNA/RNA Shield™**, see page 6. DNA/RNA Shield™ lyses cells, inactivates nucleases and infectious agents (e.g., virus, pathogens) and is ideal for safe sample storage and transport at ambient temperatures.
- **Elution Volume –** ≥ 25 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).

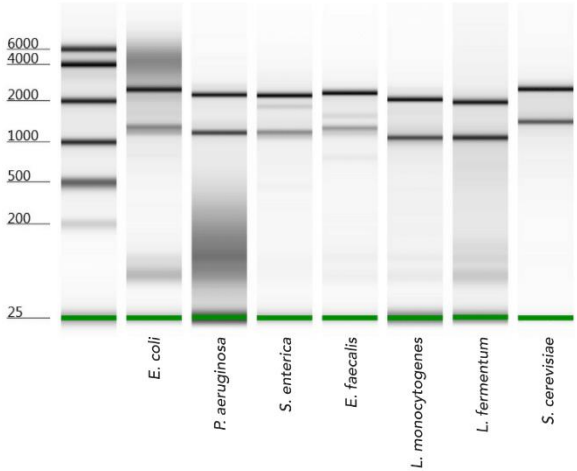
Product Description

The **Quick-RNA™ Fungal/Bacterial Miniprep Kit** provides for rapid isolation of total RNA from any tough-to-lyse gram-positive (or negative) bacteria or fungi samples. Ultra-high density **ZR BashingBeads™** are included for sample homogenization coupled with a robust buffer system, delivering total RNA (including small/microRNAs).

The **Zymo-Spin™ IIICG Column** allows for high-capacity DNA elimination and the subsequent **Zymo-Spin™ IICR Column** efficiently binds total RNA.

RNA is eluted in ≥ 25 µl and is suitable for subsequent procedures including RT-qPCR.

Efficient Recovery of RNA from Any Microbial Species



High quality total RNA is isolated from different microbial species including gram negative bacteria (E. coli, P. aeruginosa, S. enterica), gram positive bacteria (E. faecalis, L. monocytogenes, L. fermentum), and yeast (S. cerevisiae) using the **Quick-RNA™ Fungal/Bacterial** system (Agilent 2200 TapeStation™).

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. Resuspend fresh or frozen cell pellet[1] in 800 µl **RNA Lysis Buffer** and transfer mixture into a **ZR BashingBead Lysis Tube** (0.1 & 0.5 mm).

Microbes			RNA Lysis Buffer (1X)
Bacteria/Fungi (≤ 10^9) Yeast (≤ 10^8)	or	50 – 100 mg (wet weight)	800 µl

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 **Zymo-Spin™ IIICG Column**[2] in a **Collection Tube** and centrifuge. Save the flow-through!
5. To the flow-through, add an equal volume ethanol (95-100%) (1:1) and mix well.

   Example: Add 400 µl ethanol to 400 µl flow-through.
6. Transfer the mixture into a **Zymo-Spin™ IICR Column**[2] in a **Collection Tube** and centrifuge. Discard the flow-through.

   Optional: At this point, DNase I treatment can be performed. See page 7.
7. Add 400 µl **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
8. Add 700 µl **RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.
9. 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).
10. Add 50 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge. The eluted RNA can be used immediately or stored frozen.

    Alternatively, for highly concentrated RNA use ≥ 25 µl elution.

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1 Easy-to-lyse bacterial samples (gram-negative), can by lysed directly in **RNA Lysis Buffer** without mechanical homogenization/bead beating. Proceed to step 3.
2. 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 **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 **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 80 µl directly into column matrix and incubate at room temperature (20-30°C) for 15 minutes. Proceed with the purification (page 5, step 7).

DNase I Reaction Mix	
DNase I (reconstituted; 1 U/µl)[1]	5 µl
DNA Digestion Buffer	75 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™ Fungal/Bacterial Miniprep	R2014	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™ IICR Columns	C1078-50	50
Collection Tubes	C1001-50	50
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.)

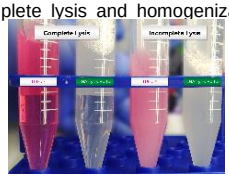
✓ For clean-up (purification) and concentration of any RNA sample. (e.g., from the aqueous phase of TRIzol® extractions) or from any enzymatic reaction (e.g., DNase I treated RNA):

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 6, page 5. - In the future, Perform in-column DNase I treatment, step 6, 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

Notes

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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.

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