



**ZYMO RESEARCH**

*The Beauty of Science is to Make Things Simple*

# INSTRUCTION MANUAL

## **Quick-RNA™ Fungal/Bacterial**

## **Miniprep Kit**

Catalog No. R2014

### **Highlights**

- Quick, 10 minute isolation of total RNA (~50 µg) from Gram-negative/positive bacteria, yeast and fungi using ultra-high density *BashingBeads™* and *Zymo-Spin™* column technologies.
- High-quality RNA eluted in ≥25 µl is ready for reverse transcription, microarray, sequencing, etc.

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

**Notes:**

<sup>1</sup> Before use, add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate (R2014).

## Product Contents

<b>Quick-RNA™ Fungal/Bacterial Miniprep Kit (Kit Size)</b>	<b>R2014 (50 preps.)</b>
<b>RNA Lysis Buffer</b>	50 ml
<b>RNA Prep Buffer</b>	25 ml
<b>RNA Wash Buffer<sup>1</sup> (concentrate)</b>	24 ml
<b>DNase/RNase-Free Water</b>	1 ml
<b>ZR BashingBead™ Lysis Tubes (0.1 &amp; 0.5 mm)</b>	50
<b>Zymo-Spin™ IICG Columns</b>	50
<b>Zymo-Spin™ IICR Columns</b>	50
<b>Collection Tubes</b>	2x 50
<b>Instruction Manual</b>	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.

**Storage Temperature** – Store all kit components (*i.e.*, buffers, columns) at room temperature.

## Specifications

- **Sample Sources** – 50-100 mg (wet weight) fungi or bacteria. This equates to approximately  $10^9$  bacterial cells and  $10^8$  yeast cells.
- **Format** – Bead beating, spin column.
- **RNA Recovery** – RNA can be eluted into small volumes,  $\geq 25 \mu\text{l}$ , allowing for a highly concentrated sample. Maximum RNA binding capacity of provided column is  $\sim 50 \mu\text{g}$ .
- **RNA Purity** – High quality total RNA ( $A_{260}/A_{280} > 1.8$ ,  $A_{260}/A_{230} > 1.8$ ) is recovered. In general, traces of DNA may be present in the eluted RNA fraction. Complete removal of DNA can be accomplished by performing an in-column DNase I digestion (page 4).
- **Compatibility** – Compatible with samples stored in RNA<sup>later</sup>™.
- **RNA Storage** – RNA is eluted with RNase-free water and can be stored at  $\leq -70 \text{ }^\circ\text{C}$ . The addition of RNase inhibitors is optional but highly recommended for prolonged storage.
- **Equipment** – Microcentrifuge, vortex and/or cell disrupter/pulverizer (optional).

Note - ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. It is not intended 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.

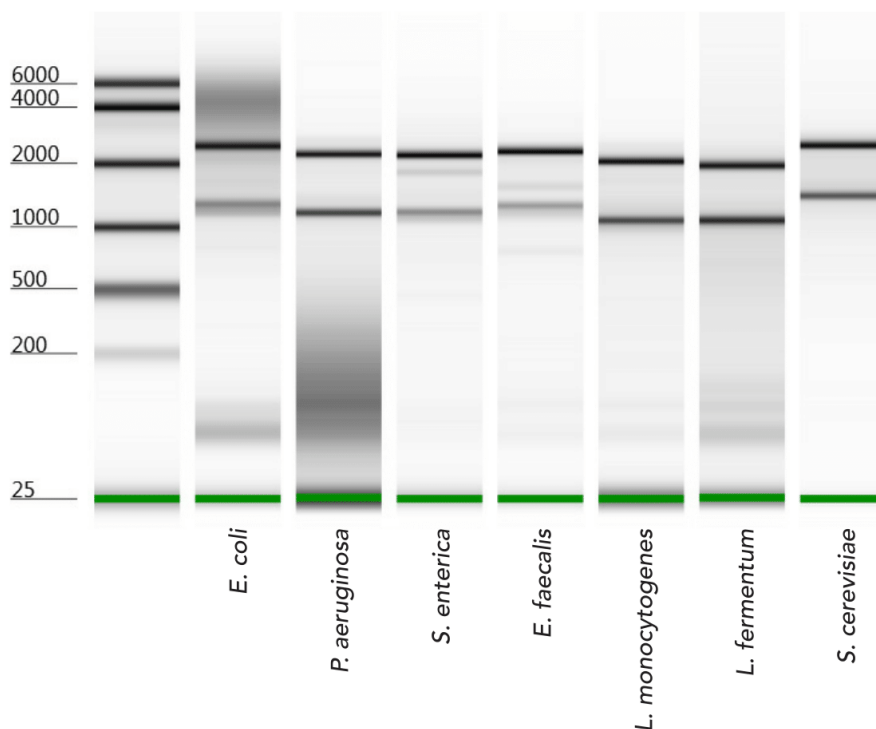
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## Product Description

The **Quick-RNA™ Fungal/Bacterial Miniprep Kit** provides for rapid isolation of RNA from pelleted *tough-to-lyse* bacterial (e.g., *Gram-positive*), yeast or fungal cells. The **Quick-RNA™ Fungal/Bacterial Miniprep Kit** employs ultra-high density **ZR BashingBeads™** for sample homogenization and a robust buffer system delivering total RNA (including small RNAs) as well as DNA removal from a variety of samples.

The **Zymo-Spin™ IICG Column** allows for high-capacity DNA elimination and the subsequent **Zymo-Spin™ IICR Column** efficiently binds total RNA. The **DNase/RNase-Free Water** eluted RNA is suitable for subsequent procedures including RT-PCR.

*RNA can be eluted into volumes as little as 25 µl in less than 10 minutes.*



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).

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

### Note:

The **Quick-RNA™ Fungal/Bacterial Miniprep Kit** can be used to isolate total RNA from *easy-to-lyse* samples (e.g., *E. coli* and other *Gram-negative* bacteria) without prior homogenization or to purify RNA directly from DNA/RNA samples (e.g., *in vitro* transcription/ translation).

For isolation of PCR-quality DNA from microbial cultures see **Quick-DNA™ Fungal/Bacterial Miniprep Kit** (Cat. #D6005).

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

**Notes:**

<sup>1</sup> Samples that do not require disintegration by **ZR BashingBead™** system (e.g. *Gram-negative* bacteria (*E. coli*)) may be lysed directly followed by Step 3.

<sup>2</sup> Processing times may be as little as 40 seconds when using high-speed cell disrupters (e.g., FastPrep®-24, or similar). See manufacturer's literature for operating information.

Disruptor Genie™ - bacterial/yeast cells: 1-2 minutes at maximum speed.

<sup>3</sup> Sample (*i.e.*, *supernatant*) and reagent volumes in this protocol can be adjusted proportionally if needed.

<sup>4</sup> To process samples >800 µl, reload the column and repeat or use a vacuum manifold.

<sup>5</sup> At this point, RNA samples can be in-column DNase I treated (page 4).

## **Buffer Preparation**

Before starting, add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate (R2014).

## **Protocol**

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

1. Resuspend a fresh or frozen cell pellet in 800 µl **RNA Lysis Buffer**<sup>1</sup> and transfer the mixture to a **ZR BashingBead™ Lysis Tube**.
2. Secure in a bead beater fitted with a 2 ml tube holder assembly and process<sup>2</sup>.
3. Centrifuge the **ZR BashingBead™ Lysis Tube** for 1 minute.
4. Transfer 400 µl supernatant<sup>3</sup> to a **Zymo-Spin™ IICG Column**<sup>4</sup> in a **Collection Tube** and centrifuge for 30 seconds. Save the flow-through!
5. Add 1 volume ethanol (95-100%) to the flow-through in the **Collection Tube** and mix well.
6. Transfer the mixture to a **Zymo-Spin™ IICR Column**<sup>4</sup> in a **Collection Tube** and centrifuge for 30 seconds<sup>5</sup>. Discard the flow-through.
7. Add 400 µl **RNA Prep Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
8. Add 700 µl **RNA Wash Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
9. 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).
10. Add 50 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge for 30 seconds.

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

The eluted RNA can be used immediately or stored at -70°C.

## **In-Column DNase I Digestion**

The DNase I digestion procedure can be performed using **DNase I Set** (E1010)<sup>1</sup>.

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

1. Following the RNA binding step (page 3, step 6), prewash the column with 400  $\mu$ l **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:

<b>DNase I</b>	5 $\mu$ l
<b>DNA Digestion Buffer</b>	75 $\mu$ l

3. Add 80  $\mu$ l 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 7).

## **Samples in DNA/RNA Shield™**

1. Bring the lysed sample to at least 800  $\mu$ l with **DNA/RNA Shield™**. Transfer the 800  $\mu$ l sample to a **ZR BashingBead™ Lysis Tube**.
2. Secure in a bead beater fitted with a 2 ml tube holder assembly and process.
3. Centrifuge the **ZR BashingBead™ Lysis Tube** for 1 minute.
4. Transfer 400  $\mu$ l supernatant to a new tube (not provided).
5. Add 1 volume **RNA Lysis Buffer** to the sample and mix well.
6. Transfer the mixture to a **Zymo-Spin™ IICG Column** in a **Collection Tube** and centrifuge for 30 seconds. Save the flow-through!
7. Continue with RNA Purification (page 3, step 5).

### **Notes:**

<sup>1</sup> 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<sub>260</sub> units/min/ml of reaction mixture at 25°C.*

**Ordering Information**

Product Description	Catalog No.	Kit Size
<b>Quick-RNA™ Fungal/Bacterial Microprep Kit</b>	R2010	50 Preps.
<b>Quick-RNA™ Fungal/Bacterial Miniprep Kit</b>	R2014	50 Preps.

For Individual Sale	Catalog No.	Amount
<b>ZR BashingBead™ Lysis Tubes (0.1 &amp; 0.5 mm)</b>	S6012-50	50
<b>RNA Lysis Buffer</b>	R1060-1-50	50 ml
	R1060-1-100	100 ml
<b>RNA Prep Buffer</b>	R1060-2-10	10 ml
	R1060-2-25	25 ml
<b>RNA Wash Buffer</b> (concentrate)	R1003-3-6	6 ml
	R1003-3-12	12 ml
	R1003-3-24	24 ml
	R1003-3-48	48 ml
<b>Zymo-Spin™ IICR Columns</b>	C1078-50	50
	C1078-250	250
<b>Zymo-Spin™ IICG Columns</b>	C1006-50-G	50
	C1006-250-G	250
<b>Collection Tubes</b>	C1001-50	50
	C1001-500	500
	C1001-1000	1000
<b>DNase/RNase-Free Water</b>	W1001-1	1 ml
	W1001-4	4 ml
	W1001-6	6 ml
	W1001-10	10 ml

**Lysis Instruments**

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

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

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