ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Quick-RNA™ Fungal/Bacterial Microprep Kit

Catalog No. **R2010**

Highlights

- Quick, 10 minute isolation of total RNA (~10 µg) from Gram-negative/positive bacteria, yeast and fungi using ultra-high density *BashingBeads*™ and Clean-Spin™ column technologies.
- High-quality RNA eluted in ≥6 µl is ready for reverse transcription, microarray, sequencing, etc.

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ZYMO RESEARCH CORP.

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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.

Product Contents

***Quick*-RNA™ Fungal/Bacterial Microprep Kit** (Kit Size)	**R2010** (50 preps.)
RNA Lysis Buffer	50 ml
RNA Prep Buffer	25 ml
RNA Wash Buffer[1] (concentrate)	24 ml
DNase/RNase-Free Water	1 ml
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	50
Zymo-Spin™ IIICG Columns	50
Zymo-Spin™ IC Columns	50
Collection Tubes	2x 50
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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] Before use, add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.

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

Specifications

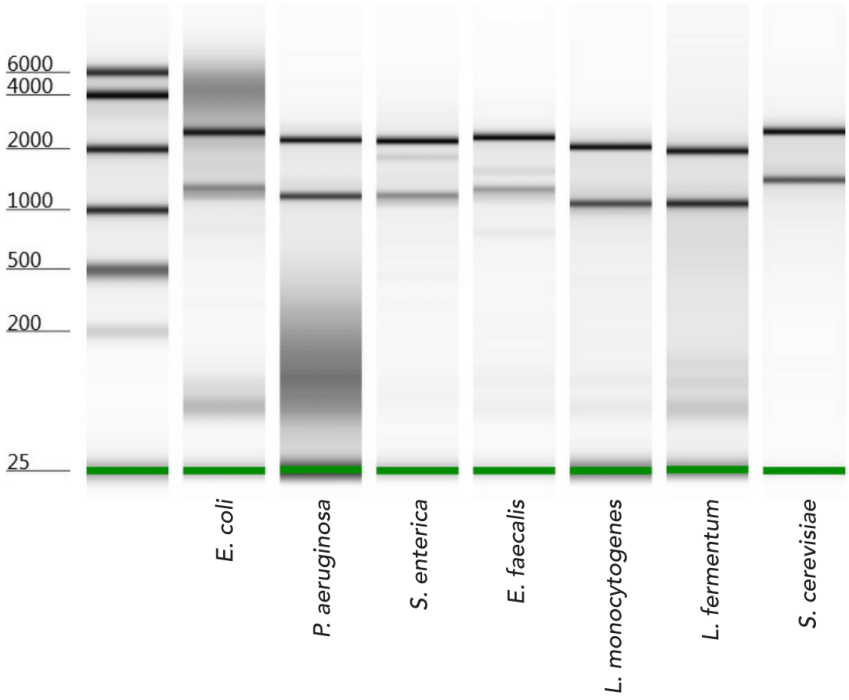
- **Sample Sources** – 10-20 mg (wet weight) fungi or bacteria. This equates to approximately $2x10^8$ bacterial cells and $2x10^7$ yeast cells.
- **Format** – Bead beating, spin column.
- **RNA Recovery** – RNA can be eluted into small volumes, ≥6 µl, allowing for a highly concentrated sample. Maximum RNA binding capacity of provided column is ~10 µg.
- **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*later*™.
- **RNA Storage** – RNA is eluted with RNase-free water and can be stored at ≤-70 ºC. The addition of RNase inhibitors is optional but highly recommended for prolonged storage.
- **Equipment** – Microcentrifuge, vortex and/or cell disrupter/pulverizer (optional).

Product Description

The ***Quick*-RNA™ Fungal/Bacterial Microprep 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 Microprep 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™ IIICG Column** allows for high-capacity DNA elimination and the subsequent **Zymo-Spin™ IC 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 6 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.

Note:

The ***Quick*-RNA™ Fungal/Bacterial Microprep 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 Microprep Kit** (Cat. **#D6007**).

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

Buffer Preparation

Before starting, 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.*

1. Resuspend a fresh or frozen cell pellet in 800 µl **RNA Lysis Buffer**[1] 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[2].
3. Centrifuge the **ZR BashingBead™ Lysis Tube** for 1 minute.
4. Transfer 400 µl supernatant[3] to a **Zymo-Spin™ IIICG Column**[4] 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™ IC Column**[4] in a **Collection Tube** and centrifuge for 30 seconds[5]. 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 15 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge for 30 seconds.

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

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

Notes:

[1] 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.

[2] 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.

[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 and repeat or use a vacuum manifold.

[5] At this point, RNA samples can be in-column DNase I treated (page 4).

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 6), 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 7).

Samples in DNA/RNA Shield™

1. Bring the lysed sample to at least 800 µl with **DNA/RNA Shield™**. Transfer the 800 µl 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 µl 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™ IIICG Column** in a **Collection Tube** and centrifuge for 30 seconds. Save the flow-through!

7. Continue with RNA Purification (page 3, step 5).

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	Catalog No.	Kit Size
***Quick*-RNA™ Fungal/Bacterial Microprep Kit**	R2010	50 Preps.
***Quick*-RNA™ Fungal/Bacterial Miniprep Kit**	R2014	50 Preps.

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

Description	Cat. No.	Amount
Disruptor Genie™, 120V w/ 2 ml tube holder assembly.	S6001-2-120	1 unit
Disruptor Genie™, 240V w/ 2 ml tube holder assembly.	S6001-2-240	1 unit
TurboMix Attachment, 2 ml 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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