ZYMO RESEARCH

RNA Purification Made Simple™

Quick-RNA™ Midiprep Kit

RNA from any sample

Highlights

- Spin-column purification of RNA (up to 1 mg) from cells and tissue.
- High-quality RNA is ready for any downstream application.

Catalog Numbers:
R1056

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

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Product Contents

Quick-RNA™ Midiprep Kit	R1056 (25 prep)
ZR RNA Buffer	100 ml
RNA Pre-Wash Buffer	12 ml
RNA Wash Buffer[1] (concentrate)	6 ml
DNase/RNase-Free Water	10 ml
Zymo-Spin™ V-E Columns w/ Reservoir	25
Collection Tubes	50
Instruction Manual	1

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature.
Before use:
1 Add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **RNA Wash Buffer** concentrate.

Specifications

- **Sample Sources –** Cells (animal, gram(-) bacteria), soft and easy-to-lyse tissue, and enzymatic reactions (e.g., DNase I treated, Proteinase K treated). Not compatible with whole blood[1], urine[1], or samples in DNA/RNA Shield™[2].
- **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™ V-E Column** yield up to 1 mg RNA.
- **Elution Volume –** ≥ 200 µl **DNase/RNase-Free Water.**
- **Equipment Needed (user provided) –** Vacuum manifold, microcentrifuge, vortex.

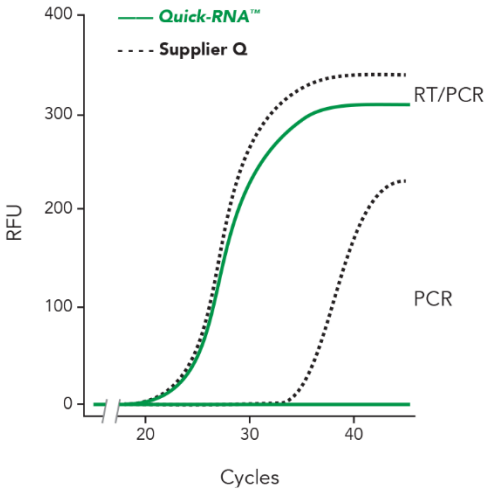
1 For RNA purification from whole-blood and urine, see the Quick-RNA Miniprep Plus Kit (R1057, R1058) or the Quick-RNA MagBead Kit (R2132, R2133).
2 For RNA purification from samples in DNA/RNA Shield™, see the Quick-RNA Microprep kit (R1050, R1051), the Quick-RNA Miniprep Kit (R1054, R1055), the Quick-RNA Miniprep Plus Kit (R1057, R1058), the Quick-RNA MagBead Kit (R2132, R2133), or the Quick-RNA 96 Kit (R1052, R1053).

Product Description

The **Quick-RNA™ Midiprep Kit** provides a quick method for the isolation of high-quality RNA of up to 1 mg from cells (animal, buccal, buffy coat) and soft, easy-to-lyse tissue.

The procedure uses unique spin-column technology that results in high-quality RNA. Simply add the provided **ZR RNA Buffer** to the sample and bind RNA directly on the **Zymo-Spin™ V-E Column**. Then wash and elute. RNA is ready for Next-Gen Sequencing, RT/qPCR, hybridization, etc.

High-Quality RNA



RNA isolated with the ***Quick*-RNA™ Kits** is DNA-free. Samples isolated with Supplier Q's kit are provided for comparison. Total RNA was isolated from 10^6 human epithelial cells. Each amplification curve represents an average of three independent isolation experiments.

Protocol

The protocol consists of: (I) Buffer Preparation, (II) Sample Preparation and (III) Total RNA Purification.

(I) Buffer Preparation

- ✓ Add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **RNA Wash Buffer** concentrate.

(II) Sample Preparation

✓ Perform all steps at room temperature and centrifugation at ≤ 500 x g for 30 seconds, unless specified.

Cells

a. **To pellet cells**: Centrifuge liquid sample at ≤ 500 x g for 1 minute and remove the supernatant. Then resuspend the cell pellet in **ZR RNA Buffer** (see table below).

b. **Adherent cells**: Remove liquid media from the culture container. Then add **ZR RNA Buffer** directly to the monolayer (see table below). Remove cells from the culture surface by scraping, pipetting, scraping, etc.

c. **Cells in suspension**: Add ≥ 3 volumes **ZR RNA Buffer** to 1 volume of liquid sample and mix well.

Mammalian	Add ZR RNA Buffer[1]
10^3 - 10^8	≥ 6 ml

To remove particulate debris, centrifuge and transfer the supernatant into a new nuclease-free tube (not provided). Then proceed to purification, page 6.

Tissue

≤ 100 mg low yield tissue (or ≤ 50 mg high yield tissue) can be mechanically homogenized in ≥ 6 ml **ZR RNA Buffer** with a mortar/pestle, dounce, syringe, tissue grinder, or bead beating (recommended). To remove particulate debris from homogenate, centrifuge and transfer the supernatant into a new nuclease-free tube (not provided). Proceed to purification, page 6.

Recommended: Use ZR BashingBead Lysis Tubes (#S6003; sold separately) and a high-speed homogenizer (e.g., MP Bio FastPrep-24, Bertin Precellys) for 30-60 seconds.

1 If the sample lysate is turbid, the volume of **ZR RNA Buffer** can be increased for complete lysis of the sample (sample lysate should appear clear with no particulate debris).

(III) Total RNA Purification

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

1. Add 1 volume[1] ethanol (95-100%) to 1 volume sample lysed in **ZR RNA Buffer** (1:1) and mix well.

   Example: Add 6 ml ethanol to 6 ml mixture (sample lysed in **ZR RNA Buffer**).

2. Transfer the mixture into a **Zymo-Spin™ V-E Column**[2] **with reservoir** mounted on a vacuum manifold and start vacuum[3].

3. Remove the reservoir and transfer the column into a **Collection Tube**. Then centrifuge the column.

4. Add 400 µl **RNA Pre-Wash Buffer** to the column and centrifuge. Discard the flow-through.

5. Add 400 µl **RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.

6. Add 400 µl **RNA Wash Buffer** and centrifuge the column for 2 minutes to ensure complete removal of the wash buffer. Then carefully, transfer the column into a nuclease-free tube (not provided).

7. Add ≥ 200 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

   The eluted RNA[4] can be used immediately or stored frozen.

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1 For total RNA without small/micro RNAs (17-200 nt), proceed directly to step 2.
2 To process samples > 400 µl, columns may be reloaded.
3 Set vacuum source at ≥ 500 mm Hg.
4 Eluted RNA can be DNase I treated using DNase I Set (cat. #E1010). For the protocol, see the RNA Clean & Concentrator-100 (cat. #R1019).

Ordering Information

Product Description	Catalog No.	Size
Quick-RNA™ Midiprep Kit	R1056	25 preps.

Individual Kit Components	Catalog No.	Amount
ZR RNA Buffer	R1020-1-100	100 ml
RNA Pre-Wash Buffer	R1020-2-50 R1020-2-100	50 ml 100 ml
RNA Wash Buffer (concentrate)	R1003-3-6 R1003-3-12	6 ml 12 ml
Zymo-Spin™ V-E Columns w/ Reservoir	C1029-25	25
Collection Tubes	C1001-50	50
DNase/RNase-Free Water	W1001-6 W1001-10	6 ml 10 ml

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.)

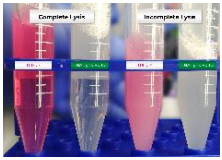
✓ For clean-up (purification) and concentration of any RNA sample. (e.g., from the aqueous phase of TRIzol® extractions) or from any enzymatic reaction (e.g., DNase I treated RNA):

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 ZR RNA 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 ZR RNA 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 ZR RNA Bufferr
DNA contamination	To remove DNA: - Perform DNase I treatment post-purification, then re-purify the treated sample. See DNase I Set #E1010 and the RNA Clean & Concentrator kit #R1019.

For technical assistance, please contact 1-888-882-9682 or email tech@zymoresearch.com

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tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682