ZYMO RESEARCH

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INSTRUCTION MANUAL

Quick-RNA™ Midiprep Kit

Catalog No. **R1056**

Highlights

- 10 minute method for isolating RNA *(*up to 1 mg*)* from a wide range of cell types and tissue samples.
- Clean-Spin™ column technology allows ultra clean RNA to be eluted into minimal volumes (≥200 µl).
- Omits the use of organic denaturants and proteases.

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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:

[1] Add 24 ml 100% ethanol (26 ml of 95% ethanol) to the 6 ml **RNA Wash Buffer** concentrate before use.

[2] For purification of RNA from whole blood, see the ***Quick*-RNA™ Whole Blood** (R1201).

Product Contents

***Quick*-RNA™ Midiprep Kit** (Kit Size)	**R1056** (25 Preps.)
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
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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.

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

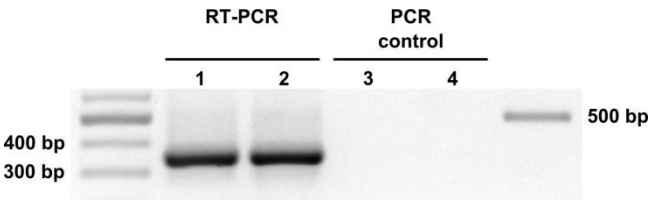
Specifications

- **Sample Sources** – Cells from culture or tissue samples, buffy coat, plasma, serum, and other biological liquids. Not compatible with whole blood.[2]
- **Sample Size** – ~10^3 to 10^8 mammalian (animal) cells.
- **Format** – 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.
- **RNA Recovery** – Up to 1 mg RNA can be eluted into ≥200 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.
- **Equipment Needed** – Vacuum manifold, microcentrifuge.

Product Description

The ***Quick*-RNA™ Midiprep Kit** is an innovative product designed for the easy, reliable, and rapid isolation of up to 1 mg total RNA from cultured cells or tissue samples. The procedure combines a unique, single-step RNA extraction/binding buffer with Clean-Spin™ column technology to yield high quality RNA in about 10 minutes. The *Quick*-RNA™ Midiprep Kit allows for the efficient recovery of total RNA from 10^3 to 10^8 cells or tissue.

The method is easy: simply add the provided **ZR RNA Buffer** to extract total RNA from the cells of interest then purify the RNA using the provided **Zymo-Spin™ V-E Columns**. The result is highly-concentrated, purified RNA that is suitable for subsequent RNA-based methods including RT-PCR, hybridization, *etc*.



PCR amplification of β-actin transcript (353 bp fragment shown) following RNA isolation from human epithelial cells (HCT 116) with the ***Quick*-RNA™ Midiprep Kit**: RT-PCR (lane 1, 2), PCR negative control (RNA template; lane 3, 4).

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

***Quick*-RNA™** - *Fast-Spin* column and plate technology overview

	***Quick*-RNA™ Microprep Kit**	***Quick*-RNA™ Miniprep Kit**	***Quick*-RNA™ Midiprep Kit**	***Quick*-RNA™ 96 Kit**
Format	Zymo-Spin™ IC Column	Zymo-Spin™ IIICG Column	Zymo-Spin™ V-E Column	Silicon-A™ Plate
Catalog	R1050/R1051	R1054/R1055	R1056	R1052/R1053
Preps	50/200	50/200	25	2x 96/4x 96
Input	~1-10^6 cells	~10^2-10^7 cells	~10^3-10^8 cells	~1-10^6 cells
Binding	~10 µg	~100 µg	~1 mg	~10 µg/well

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

Clean-Spin™ column technology efficiently removes the majority of DNA during RNA purification and is satisfactory for most RNA-based applications. However, if necessary, complete removal of DNA can be achieved by performing a DNase I digestion. See the **RNA Clean & Concentrator™**.

Notes:

[1] In order to lyse samples completely, the amount of the **ZR RNA Buffer** should be adjusted (*i.e.,* more buffer can be added).

[2] Total RNA including small RNAs ≥17nt is recovered. **Zymo-Spin™** columns may be reloaded.

[3] Total RNA without small RNAs (*e.g.*, tRNAs and microRNAs) is recovered

[4] Set vacuum source at ≥500 mm Hg.

[5] To maximize RNA yield, increase the elution volume (≥200 µl) and/or repeat the elution.

Buffer Preparation

Before starting, add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **RNA Wash Buffer** concentrate.

Protocol

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

1. **Sample Homogenization and Cell Lysis:** Follow an applicable method (A-D).

   A. Adherent Cells: Cells can be lysed directly in the culture container by removing liquid medium and adding **ZR RNA Buffer**[1] directly to the monolayer (e.g., 3 ml for $5x10^6$ cells). Remove cells from culture surface by pipetting, scraping, *etc*.

   B. Cells in Suspension: Pellet cells by gentle centrifugation (e.g., 5 minutes at 500 x g). Remove the supernatant completely and resuspend the cell pellet in 3 ml **ZR RNA Buffer**[1]. Vortex briefly.

   C. Solid Tissue Samples: Add 3 ml ZR RNA Buffer[1] to fresh or frozen tissue (up to ~100 mg) and homogenize the sample (e.g., using a Dounce or similar homogenizer). **Important!** To eliminate tissue debris, if present, sample lysates may be spun at ≤500 x g for 1 minute.

   D. Liquid Samples: Add 3 volumes of **ZR RNA Buffer**[1] to every volume of sample (e.g., 3 ml of buffer to 1 ml sample).

For total RNA including small RNAs[2]	**For total RNA without small RNAs**[3]
Add 1 volume ethanol (95-100%) to the lysate and mix thoroughly by pipetting.	Go directly to Step 2.

2. Transfer lysate into the **Zymo-Spin™ V-E Column with Reservoir** mounted on a vacuum manifold and start vacuum[4].

3. Remove the reservoir and transfer column into a **Collection Tube**. Centrifuge the column for 30 seconds.

4. Add 400 µl **RNA Pre-Wash Buffer** to the column and centrifuge for 1 minute. Discard the flow-through.

5. Add 400 µl **RNA Wash Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through. Repeat this step.

6. Centrifuge the column for 2 minutes in the emptied collection tube to ensure complete removal of the wash buffer. Then, carefully transfer the column into an RNase-free tube (not provided).

7. Add ≥200 µl **DNase/RNase-Free Water**[5] directly to the column matrix and let stand at room temperature for 1 minute. Centrifuge for 1 minute.

   The eluted RNA can be used immediately or stored at ≤-70 ºC.

Ordering Information

Product Description	Input	Binding	Catalog No.	Kit Size
***Quick*-RNA™ Microprep Kit**	~1-10^6 cells	~10 µg	R1050 R1051	50 Preps. 200 Preps.
***Quick*-RNA™ Miniprep Kit**	~10^2-10^7 cells	~100 µg	R1054 R1055	50 Preps. 200 Preps.
***Quick*-RNA™ Miniprep Plus Kit**	~10^2-10^7 cells	~100 µg	R1057T R1057 R1058	10 Preps. 50 Preps. 200 Preps.
***Quick*-RNA™ Midiprep Kit**	~10^6-10^8 cells	~1 mg	R1056	25 Preps.
***Quick*-RNA™ 96 Kit**	~1-10^6 cells	~10 µg/well	R1052 R1053	2x 96 Preps. 4x 96 Preps.

For Individual Sale	Catalog No.	Amount
ZR RNA Buffer	R1020-1-50 R1020-1-100 R1020-1-200	50 ml 100 ml 200 ml
RNA Pre-Wash Buffer	R1020-2-12 R1020-2-25 R1020-2-50 R1020-2-100	12 ml 25 ml 50 ml 100 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™ V-E Columns w/ Reservoir	C1029-25	25
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

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