ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Quick-RNA™ Miniprep Kit

Catalog Nos. **R1054 & R1055**

Highlights

- High-quality total RNA (including small RNAs) from a wide range of samples.
- You can opt to isolate small and large RNAs in separate fractions.
- *DNA-free* RNA is ready for use in any downstream application. *DNase I included.*

Contents

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Satisfaction of all Zymo Research products is guaranteed. If you are dissatisfied with this product please contact us.

Product Contents

***Quick*-RNA™ Miniprep Kit** (Kit Size)	**R1054** (50 Preps.)	**R1055** (200 Preps.)
RNA Lysis Buffer	50 ml	2x 100 ml
RNA Prep Buffer	25 ml	100 ml
RNA Wash Buffer[1] (concentrate)	24 ml	2x 48 ml
DNase/RNase-Free Water	6 ml	30 ml
DNase I[2] (lyophilized)	1	4
DNA Digestion Buffer	4 ml	16 ml
Spin-Away™ Filters	50	200
Zymo-Spin™ IIICG Columns	50	200
Collection Tubes	100	400
Instruction Manual	1	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.

[1] Before use, add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate or 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate.

[2] Prior to use, reconstitute the lyophilized **DNase I** as indicated on the vial prior to use. Store frozen aliquots.

Specifications

Some difficult-to-lyse samples may require mechanical or enzymatic homogenization. For assistance, contact us at tech@zymoresearch.com.

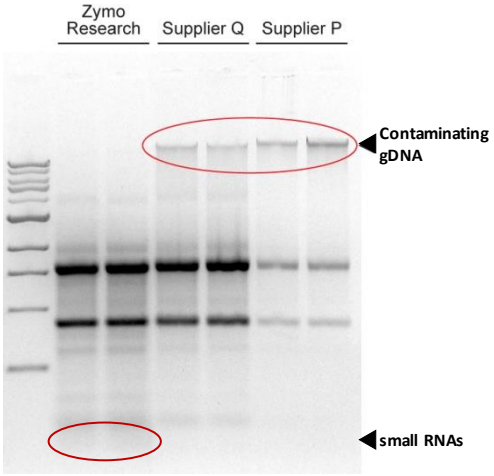
Use the ***Quick*-RNA™ Microprep Kit** (Cat. Nos. R1050, R1051) for up to 10 µg RNA from 1-10^6 cells.

- **Sample Sources** – Cells or tissue samples, yeast, plant or bacteria. Compatible with DNA/RNA Shield™ and RNA*later*™.
- **Sample Storage** – Samples homogenized in RNA Lysis Buffer are stable and can be stored frozen prior to purification.
- **Sample Size** – Up to 10^7 cells or 50 mg tissue.
- **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 100 µg RNA can be eluted into ≥50 µl RNase-free water allowing for a highly concentrated sample.
- **RNA Storage** – RNA is eluted with RNase-free water and can be stored frozen. RNase inhibitors can be included for prolonged storage.
- **Equipment Needed** – Microcentrifuge.

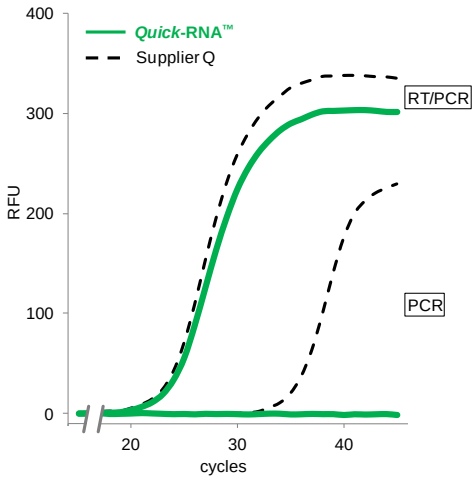
Product Description

The ***Quick*-RNA™ Miniprep Kit** is an innovative product designed for the easy, reliable, and rapid isolation of DNA-free RNA from a wide range of cell *(up to 10^7)* and tissue samples *(up to 50 mg)*. The procedure combines a unique buffer system with Clean-Spin™ column technology to yield high quality total RNA *(including small RNAs 17-200 nt)* in about 10 minutes.

The procedure is simple. Add the provided **RNA Lysis Buffer** to a sample, and then purify the RNA using the **Zymo-Spin™ Columns**. The result is highly-concentrated, *DNA-free* RNA that is suitable for RT-PCR, hybridization, sequencing *etc*. In addition, the kit can be used for the enrichment of small and large RNAs into separate fractions (page 5).



The ***Quick*-RNA™ Miniprep Kit** yields high quality total RNA. High levels of genomic DNA contamination are present in the preps from Suppliers Q & P but not with the ***Quick*-RNA™ Miniprep Kit**. Total RNA was isolated from human epithelial cells (sans DNase treatment).



RNA isolated with the ***Quick*-RNA™ Miniprep Kit** is DNA-free. Samples isolated with Supplier Q's kit are provided for comparison. Total RNA was isolated from 10^6 human epithelial cells (with in-column DNase treatments for both kits). Each amplification curve represents an average of three independent isolation experiments.

RNA MiniPrep Kit Comparison

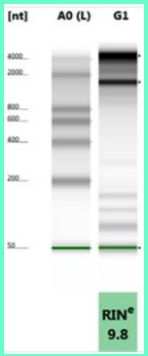
	Quick-RNA™	Supplier Q
Small RNA (≥17 nt) recovery	**YES**	NO
DNase I included	**YES**	NO
gDNA removal column included	**YES**	NO

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

Notes:

Use the **Direct-zol™ RNA Miniprep Kit** (Cat. Nos. R2050, R2051, R2052, R2053) for isolation of RNA directly (without phase separation) from samples in Trizol®, *etc.*

Use the **DNA/RNA Shield™** for safe sample storage and transport at ambient temperatures.



The ***Quick*-RNA™** kits yield high quality RNA as indicated by the RIN (RNA Integrity Number; 2200 TapeStation, Agilent).

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 (R1054) or 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate (R1055).

✓ Reconstitute the lyophilized **DNase I** as indicated on the vial prior to use and store aliquots at -20°C.

Protocols

The RNA isolation consists of three steps: (I) Sample Lysis/Homogenization, (II) Sample Clearing and gDNA Removal and (III) RNA Purification.

All steps should be performed at room temperature (20-30 ºC).

I. Sample Lysis/Homogenization

Notes:

Samples homogenized in **RNA Lysis Buffer** can be stored frozen for processing at a later time.

Recommended **RNA Lysis Buffer** volumes

RNA Lysis Buffer	300 µl	600 µl
Cells	Up to 5 x 10^6	>5 x 10^6
Tissue	<20 mg	≤50 mg

Adherent Cells

Lyse cells directly in the culture container by removing liquid medium and adding **RNA Lysis Buffer** directly to the monolayer.

Cells in Suspension

Pellet cells *(≤500 x g)*, remove the supernatant completely then resuspend the cell pellet in **RNA Lysis Buffer**. Vortex briefly.

Tissue and Tough-to-Lyse Samples

Fresh or frozen tissue (animal, plant, insect, yeast or bacteria) can be mechanically homogenized (e.g., **ZR BashingBead™ Lysis Tubes**) directly in the **RNA Lysis Buffer**.

Alternatively, tough-to-lyse tissue samples can be Proteinase K treated (page 5).

ZR Bashing Bead™ Lysis Tubes are available separately (Cat. Nos. S6002, S6003).

Processing plant tissue and other samples containing polyphenolics, humic acids, melanin, *etc.* may require use of the **OneStep™ PCR Inhibitor Removal Kit** (Cat. No. D6030).

Use the **DNA/RNA Shield™** for safe sample storage and transport at ambient temperatures.

Liquids/Reaction Clean-up

DNase-treated RNA, labeling and *in vitro* transcription reactions can be processed directly by adding 4 volumes of **RNA Lysis Buffer** to each volume of sample (4:1) then mixing well.

Samples in DNA/RNA Shield™

Bring samples homogenized and stored in **DNA/RNA Shield™** to room temperature (20-30 ºC). Then add 1 volume **RNA Lysis Buffer** (1:1), mix and proceed with Sample Clearing step.

Samples in DNA/RNA Shield™ can be Proteinase K treated (page 5).

Samples in RNA*later*™

To process cells or liquids in RNA*later*™ (without reagent removal): Add 1 volume of RNase-free water or PBS to the sample (1:1). Then add 4 volumes **RNA Lysis Buffer** (4:1) and mix.

Alternatively, remove the RNA*later*™, then proceed with Sample Lysis/Homogenization according to the sample type.

II. Sample Clearing and gDNA Removal

The following is recommended for cells and tissue (animal/plant) but can be omitted for cell-free liquids and low input samples (≤10^5 cells).

1. Clear lysate by centrifugation at ≥10,000 x *g* for 1 minute.
2. Transfer the supernatant into a **Spin-Away™ Filter** (**yellow**) in a **Collection Tube** and centrifuge at ≥10,000 x *g* for 1 minute to remove the majority of gDNA.

   Save the flow-through for RNA Purification!

III. RNA Purification

All centrifugation steps should be performed between 10,000-16,000 *x g.*

1. Add 1 volume ethanol (95-100%) to the sample in **RNA Lysis Buffer** (1:1). Mix well.
2. Transfer the mixture to a **Zymo-Spin™ IIICG Column**[1] (**green**) in a **Collection Tube** and centrifuge for 30 seconds. Discard the flow-through.
3. **In-column DNase I Treatment** (optional)

   This step can be used for trace DNA removal.

   a. Prewash the column with 400 µl **RNA Wash Buffer**. Centrifuge for 30 seconds. Discard the flow-through.

   b. 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[2]	5 µl
DNA Digestion Buffer	75 µl

   c. Add 80 µl **DNase I Reaction Mix** directly to the column matrix. Incubate at room temperature (20-30 ºC) for 15 minutes. Then centrifuge for 30 seconds.

4. Add 400 µl **RNA Prep Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
5. Add 700 µl **RNA Wash Buffer** to the column and centrifuge for 30 seconds. 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. Transfer the column carefully into an RNase-free tube (not provided).
7. Add 100 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge for 30 seconds.

   Alternatively, for highly concentrated RNA use ≥50 µl elution.

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

Notes:

[1] To process samples >700 µl, **Zymo-Spin™** columns may be reloaded.

[2] 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.

Purification of Small and Large RNAs into Separate Fractions

This procedure is compatible with animal cell inputs (up to 10^6) or previously isolated RNA only.

All centrifugation steps should be performed between 10,000-16,000 x *g*.
This protocol requires two columns (per prep).

1. Mix an equal volume of **RNA Lysis Buffer** and ethanol (95-100%).

   Example: Mix 50 µl buffer and 50 µl ethanol.

2. Add 2 volumes of the buffer/ethanol to an RNA sample[1] or 300 µl buffer/ethanol to a cell pellet and mix.

   Example: Mix 100 µl buffer/ethanol and 50 µl sample.

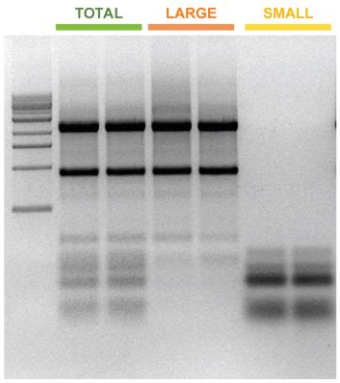
3. Transfer the mixture[2] to the **Zymo-Spin™ Column** and centrifuge for 30 seconds. **Save the flow-through!**

Column: RNAs >200 nt	**Flow-through**: RNAs 17-200 nt
4. Continue to step 5.	Add 1 volume ethanol and mix. Example: Add 150 µl ethanol to 150 µl flow-through. Transfer the mixture to a new column and centrifuge for 30 seconds. Discard the flow-through.

5. Add 400 µl **RNA Prep Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
6. Add 700 µl **RNA Wash Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
7. Add 400 µl **RNA Wash Buffer** and centrifuge the column for 2 minutes to ensure complete removal of the wash buffer. Transfer the column carefully into an RNase-free tube (not provided).
8. Add 100 µl **DNase/RNase-Free Water** directly to the column matrix, then centrifuge at top speed for 30 seconds.

   Alternatively, for highly concentrated RNA use ≥50 µl elution.

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



Total RNA (>17 nt), large (>200 nt) or small RNAs (17-200 nt) are effectively partitioned and purified with the ***Quick*-RNA™** kit.

Proteinase K Digestion

Example:	up to 5 mg solid tissue or 10^6 animal cells in DNA/RNA Shield™	95 µl
	2X Digestion Buffer[3]	95 µl
	Proteinase K[4]	≥6 U

Prepare a Proteinase K reaction mix (see example above, scale-up as necessary). Incubate at 55°C for 30 minutes (*e.g.,* pelleted white blood cells) or 1-3 hours (solid tissue). Then add 1 volume **RNA Lysis Buffer** and proceed to Sample Clearing and gDNA Removal (page 4).

Notes:

[1] Adjust the sample volume to 50 µl (minimum).

[2] **Zymo-Spin™** columns may be reloaded to process samples >700 µl,.

[3] **2X Digestion Buffer** (Cat. No. D3050-1-5 and D3050-1-20).

[4] **Proteinase K** (Cat. No. D3001-2-5 and D3001-2-20).

One unit of enzyme will hydrolyze urea-denatured hemoglobin to produce 1.0 µmole of tyrosine per minute at pH 7.5 at 37°C.

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
RNA Lysis Buffer	R1060-1-50 R1060-1-100	50 ml 100 ml
RNA Prep Buffer	R1060-2-10 R1060-2-25 R1060-2-100	10 ml 25 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
DNase I (lyophilized) (250 U supplied with DNA Digestion Buffer, 4 ml)	E1010	1 set
Spin-Away™ Filter	C1006-50-F C1006-250-F	50 250
Zymo-Spin™ IIICG Column	C1006-50-G C1006-250-G	50 250
Collection Tube	C1001-50 C1001-500 C1001-1000	50 500 1000
DNase/RNase-Free Water	W1001-1 W1001-6 W1001-10	1 ml 6 ml 10 ml

RNA MADE SIMPLE

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