ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Quick-RNA™ 96 Kit

Catalog Nos. **R1052 & R1053**

Highlights

- High throughput (96-well) isolation of total RNA (including small RNAs) from a wide range of samples - single to 10^6 cells.
- *DNA-free* RNA 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 call 1-888-882-9682.

For assistance, contact us at tech@zymoresearch.com.

Product Contents

Quick-RNA™ 96 Kit (Kit Size)	R1052 (2x 96 Preps.)	R1053 (4x 96 Preps.)	Storage Temperature
RNA Lysis Buffer	2x 100 ml	4x 100 ml	Room Temp.
RNA Prep Buffer	100 ml	2x 100 ml	Room Temp.
RNA Wash Buffer[1] (concentrate)	2x 48 ml	4x 48 ml	Room Temp.
DNase/RNase-Free Water	10 ml	30 ml	Room Temp.
DNase I[2] (lyophilized)	4	8	-20ºC (reconstituted)
DNA Digestion Buffer	16 ml	2x 16 ml	Room Temp.
Silicon-A™ Plate	2	4	Room Temp.
Collection Plate	2	4	Room Temp.
Elution Plate	2	4	-20ºC (reconstituted)
Cover Foil	2	4	Room Temp.
Instruction Manual	1	1	Room Temp.

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] Add 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate before use.

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

Specifications

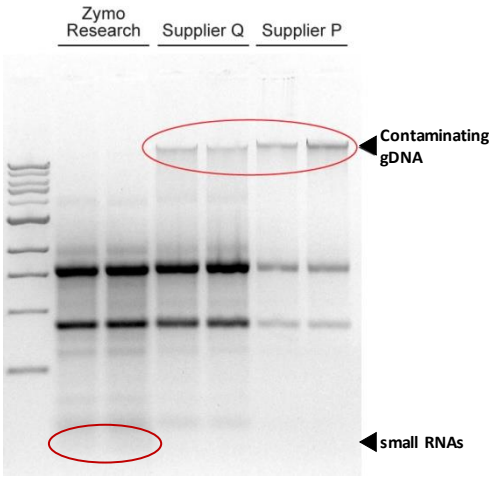
- **Sample Types** – Cells or tissue samples, yeast, plant, bacteria, buccal cells, buffy coat, plasma, serum, and other biological liquids. *Compatible with DNA/RNA Shield™ and RNAlater™.*
- **Sample Storage** – Samples homogenized in RNA Lysis Buffer are stable and can be stored frozen prior to purification.
- **Sample Size** – Up to 10^6 cells or 5 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.
- **Yield** – Up to 10 µg RNA can be eluted into ≥25 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.
- **Required Equipment** – Centrifuge/rotor compatible with 96-well plates.

Notes:

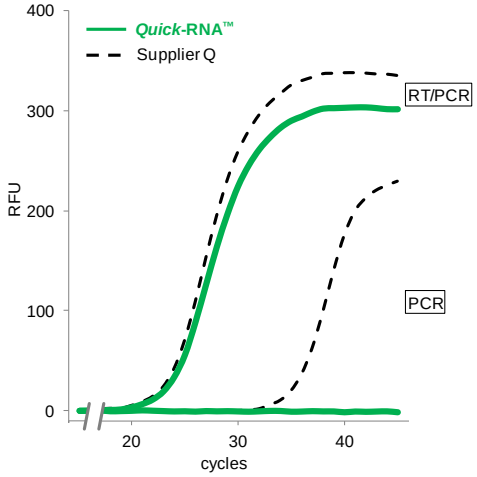
This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

Product Description

The ***Quick*-RNA™ 96 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 10^6)* and tissue samples *(up to 5 mg)*. The procedure combines a unique buffer system with Zymo-Spin™ plate technology to yield high quality total RNA *(including small RNAs ~17-200 nt)* in about 30 minutes.
The procedure is simple: Add the provided **RNA Lysis Buffer** to a sample, then purify the RNA using the provided **Silicon-A™ Plate**. The result is highly-concentrated, *DNA-free* RNA that is suitable for subsequent RNA-based methods including RT-PCR, hybridization, sequencing *etc*.



The ***Quick*-RNA™ 96 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™ 96 Kit**. Total RNA was isolated from human epithelial cells (sans DNase treatment).



RNA isolated with the ***Quick*-RNA™ 96 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.

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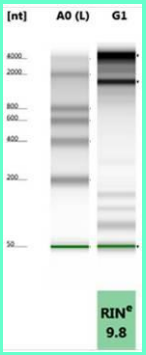
Notes:

Use the **Direct-zol™-96 RNA Kit** (Cat. Nos. R2054, R2055, R2056, R2057) for isolation of RNA directly (without phase separation) from samples in Trizol®, *etc.*

Use the **DNA/RNA Shield™** (Cat. Nos. R1100-50, R1100-250) for safe sample storage and transport at ambient temperatures.



The ***Quick*-RNA™** kits yield high quality RNA with high "RNA Integrity Numbers" (2200 TapeStation, Agilent).

Ensure the RNA isolation procedure is performed in an RNase-free environment.

Reagent Preparation

- ✓ Before starting, add 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate.
- ✓ 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 (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.

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.

Recommended **RNA Lysis Buffer** volumes

RNA Lysis Buffer	100 µl	300 µl
Cells	Up to 10^5	Up to 10^6
Tissue	-	Up to 5 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).

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). 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 RNAlater™, then proceed with Sample Lysis/Homogenization according to the sample type.

II. Sample Clearing

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).

For particulate removal, centrifuge lysates at ≥12,000 x *g* for 1 minute. Then transfer up to 300 µl of the supernatant into an RNase-free tube/plate (*not provided*).

III. RNA Purification

All centrifugation steps should be performed at ≥2,500 *x g.*

1. Add 1 volume ethanol (95-100%) to sample in the **RNA Lysis Buffer** [1:1] and mix well.
2. Transfer the mixture to a **Silicon-A™ Plate**[1] mounted on a **Collection Plate** and centrifuge for 5 minutes. Discard the flow-through.
3. **In-column DNase I Treatment** (optional)

   This step can be used for trace DNA removal.

   a. Add 400 µl/well **RNA Wash Buffer** and centrifuge for 5 minutes. 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	35 µl

   c. Add 40 µl **DNase I Reaction Mix** directly to the matrix. Incubate the plate at room temperature (20-30 ºC) for 15 minutes. Then centrifuge for 5 minutes.
4. Add 400 µl **RNA Prep Buffer** to the plate and centrifuge for 5 minutes. Discard the flow-through.
5. Add 500 µl **RNA Wash Buffer** to the plate and centrifuge for 5 minutes. Discard the flow-through. **Repeat this step.**
6. Mount the **Silicon-A™ Plate** onto an **Elution Plate**, and add ≥25 µl **DNase/RNase-Free Water**[3] directly to the matrix, then centrifuge for 5 minutes.

   The eluted RNA can be used immediately or stored frozen. Use the **Cover Foil** to prevent evaporation.

Notes:

[1] To process samples >600 µl, **Silicon-A™ Plate** 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.

[3] To maximize RNA yield, preheat the DNase/RNase-Free Water to 95º C, increase the elution volume and/or repeat the elution.

Notes:

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

[2] **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.

Proteinase K Digestion

Example:	up to 5 mg solid tissue or 10^6 animal cells in DNA/RNA Shield™	95 µl
	2X Digestion Buffer[1]	95 µl
	Proteinase K[2]	≥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 (page 4).

Ordering Information

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

For Individual Sale	Amount	Catalog No.
RNA Lysis Buffer	50 ml 100 ml	R1060-1-50 R1060-1-100
RNA Prep Buffer	10 ml 25 ml 100 ml	R1060-2-10 R1060-2-25 R1060-2-100
RNA Wash Buffer (concentrate)	6 ml 12 ml 24 ml 48 ml	R1003-3-6 R1003-3-12 R1003-3-24 R1003-3-48
DNase I (lyophilized) (250 U supplied with DNA Digestion Buffer, 4 ml)	1 set	E1010
Silicon-A™ Plate	2	C2001
Collection Plate	2	C2002
Elution Plate	2	C2003
Cover Foil	2 4	C2007-2 C2007-4
DNase/RNase-Free Water	1 ml 6 ml 10 ml	W1001-1 W1001-6 W1001-10

RNA MADE SIMPLE

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