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 call 1-888-882-9682.

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

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.

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.

Note - ™ Trademarks of Zymo Research Corporation. RNA*later*™ is a trademark of Ambion, Inc.

## Product Contents

| ***Quick*-RNA™ Miniprep Kit** (Kit Size) | **R1054** (50 Preps.) | **R1055** (200 Preps.) | **Storage Temperature** |
|---|---|---|---|
| **RNA Lysis Buffer** | 50 ml | 2x 100 ml | Room Temp. |
| **RNA Prep Buffer** | 25 ml | 100 ml | Room Temp. |
| **RNA Wash Buffer**[1] (concentrate) | 24 ml | 2x 48 ml | Room Temp. |
| **DNase/RNase-Free Water** | 6 ml | 30 ml | Room Temp. |
| **DNase I**[2] (lyophilized) | 1 | 4 | Room Temp. |
| **DNA Digestion Buffer** | 4 ml | 16 ml | Room Temp. |
| **Spin-Away™ Filters** | 50 | 200 | Room Temp. |
| **Zymo-Spin™ IIICG Columns** | 50 | 200 | Room Temp. |
| **Collection Tubes** | 100 | 400 | 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] 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 at -20°C.

## Specifications

- **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.

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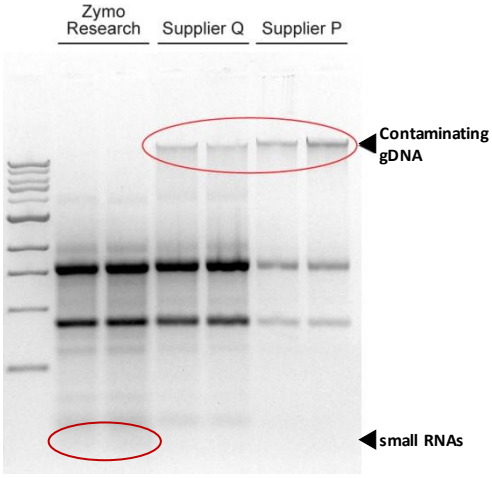
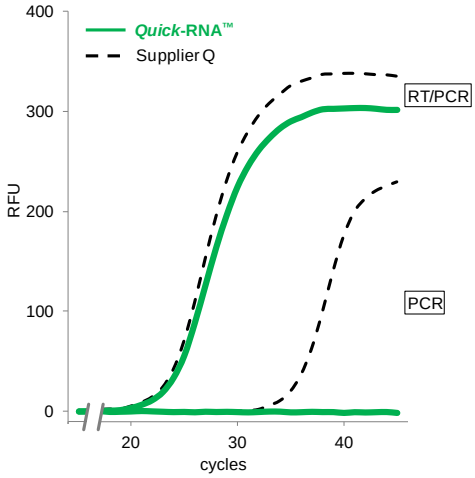
## Product Description

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**

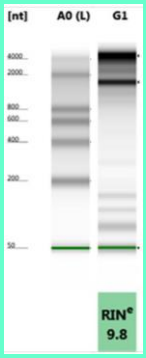
| | Quick-RNA™ | Supplier Q |
|---|---|---|
| Small RNA (≥17 nt) recovery | **YES** | NO |
| DNase I included | **YES** | NO |
| gDNA removal column included | **YES** | NO |

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.

## Reagent 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.

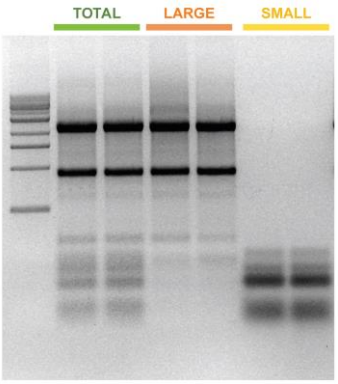
3. Transfer the mixture[2] to the **Zymo-Spin™ Column** and centrifuge for 30 seconds. **Save the flow-through!**

| | **Column**: RNAs >200 nt | **Flow-through**: RNAs 17-200 nt |
|---|---|---|
| 4. | Continue to step 5. | Add 1 volume ethanol and mix.<br>Example: Add 150 µl ethanol to 150 µl flow-through.<br>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 | Kit Size | Catalog No. |
|---|---|---|---|---|
| ***Quick*-RNA™ Microprep Kit** | ~1-$10^6$ cells | ~10 µg | 50 Preps.<br>200 Preps. | R1050<br>R1051 |
| ***Quick*-RNA™ Miniprep Kit** | ~$10^2$-$10^7$ cells | ~100 µg | 50 Preps.<br>200 Preps. | R1054<br>R1055 |
| ***Quick*-RNA™ Miniprep Plus Kit** | ~$10^2$-$10^7$ cells | ~100 µg | 10 Preps.<br>50 Preps.<br>200 Preps. | R1057T<br>R1057<br>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.<br>4x 96 Preps. | R1052<br>R1053 |

| For Individual Sale | Amount | Catalog No. |
|---|---|---|
| **RNA Lysis Buffer** | 50 ml<br>100 ml | R1060-1-50<br>R1060-1-100 |
| **RNA Prep Buffer** | 10 ml<br>25 ml<br>100 ml | R1060-2-10<br>R1060-2-25<br>R1060-2-100 |
| **RNA Wash Buffer** (concentrate) | 6 ml<br>12 ml<br>24 ml<br>48 ml | R1003-3-6<br>R1003-3-12<br>R1003-3-24<br>R1003-3-48 |
| **DNase I** (lyophilized)<br>(250 U supplied with DNA Digestion Buffer, 4 ml) | 1 set | E1010 |
| **Spin-Away™ Filter** | 50<br>250 | C1006-50-F<br>C1006-250-F |
| **Zymo-Spin™ IIICG Column** | 50<br>250 | C1006-50-G<br>C1006-250-G |
| **Collection Tube** | 50<br>500<br>1000 | C1001-50<br>C1001-500<br>C1001-1000 |
| **DNase/RNase-Free Water** | 1 ml<br>6 ml<br>10 ml | W1001-1<br>W1001-6<br>W1001-10 |

# RNA MADE SIMPLE

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