



ZYMO RESEARCH

RNA
Purification
Made Simple

Quick-RNA™ 96 Kit

RNA from any sample

Highlights

- 96-well spin-plate purification of total RNA (including small/microRNAs) from cells and tissue.
- DNA-free RNA is ready for Next-Gen Sequencing, RT/qPCR, etc. *DNase I is included.*

Catalog Numbers:

R1052, R1053



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



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Product Contents

Quick-RNA™ 96 Kit	R1052 (2 x 96 prep)	R1053 (4 x 96 prep)
RNA Lysis Buffer	100 ml (x2)	100 ml (x4)
RNA Prep Buffer	100 ml	100 ml (x2)
RNA Wash Buffer ¹ (concentrate)	48 ml (x2)	48 ml (x4)
DNase/RNase-Free Water	10 ml	30 ml
DNase I ² (lyophilized)	250 U (x4)	250 U (x8)
DNA Digestion Buffer	16 ml	16 ml (x2)
Silicon A™ Plate	2	4
Collection Plate	2	4
Elution Plate	2	4
Cover Foil	2	4
Instruction Manual	1	1

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

Before use:

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

2 Reconstitute lyophilized **DNase I** with **DNase/RNase-Free Water**, mix by gentle inversion and store frozen aliquots:

#E1009-A (250 U), add 275 µl water

Specifications

- **Sample Sources** – Cells (animal, buccal, buffy coat, gram(-) bacteria) and soft, easy-to-lyse tissue, plasma, serum, etc. Not compatible with whole-blood¹ and urine² samples.
- **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. Trace DNA can be removed by DNase I digestion (page 6).
- **Binding Capacity – Silicon A™ Plate** yield up to 10 μg RNA/well.
- **Compatibility** – For samples stored in preservation reagents: **DNA/RNA Shield™**, RNAprotect[®], Allprotect[®], Universal transport medium/viral transport medium (UTM[®]/VTM[®]) and RNAlater™.
- **Elution Volume** – ≥ 25 μl **DNase/RNase-Free Water**.
- **Equipment Needed (user provided)** – Centrifuge/rotor compatible with 96-well plates.

¹ For RNA purification from whole-blood, see the Quick-RNA Miniprep Plus Kit (R1057, R1058).

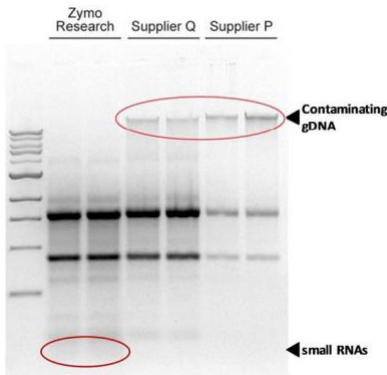
² For urine, DNA/RNA can be isolated with the Quick-DNA/RNA MagBead Kit (R2130, R2131).

Product Description

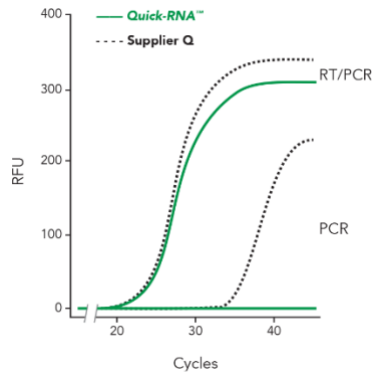
The **Quick-RNA™ 96 Kit** provides a quick method for the isolation of high-quality total RNA (≥ 17 nt) from cells (animal, buccal, buffy coat, gram(-) bacteria) and soft, easy-to-lyse tissue.

The procedure combines a unique buffer system and spin-plate technology that results in high-quality total RNA (including small/microRNAs) and is ready for Next-Gen Sequencing, RT/qPCR, hybridization, etc.

High-Quality, DNA-free RNA



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



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 (with in-column DNase treatments for both kits). 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 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate.
- ✓ Reconstitute lyophilized **DNase I** with **DNase/RNase-Free Water**, mix by gentle inversion and store frozen aliquots: **#E1009-A (250 U)**, add 275 μ l **water**

(II) Sample Preparation

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

Samples stabilized and stored in DNA/RNA Shield™ (cells, tissue, swab, etc.)

If frozen, thaw homogenized sample in **DNA/RNA Shield™** to room temperature (20-30°C). Mix well by vortex. Then add an equal volume of **RNA Lysis Buffer** (1:1) and mix well. Proceed to purification, page 6.

Cells

- To pellet cells:** Centrifuge liquid sample at ≤ 500 x g for 1 minute and remove the supernatant. Then resuspend the cell pellet in **RNA Lysis Buffer** (see table below).
- Adherent cells:** Remove liquid media from the culture container. Then add **RNA Lysis Buffer** directly to the monolayer (see table below). Remove cells from the culture surface by scraping, pipetting, scraping, etc.
- Cells in suspension:** Add ≥ 3 volumes **RNA Lysis Buffer** to 1 volume of liquid sample and mix well.

Mammalian	Gram(-) bacteria	Add RNA Lysis Buffer
$\leq 10^5$	-	$\geq 100 \mu\text{l}$
$\leq 10^6$	$\leq 10^8$	$\geq 300 \mu\text{l}$

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

Tissue¹

≤ 10 mg low yield tissue (or ≤ 5 mg high yield tissue) can be mechanically homogenized in $\geq 300 \mu\text{l}$ **RNA Lysis 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 Tissue can be Proteinase K treated prior to adding RNA Lysis Buffer (page 9).

(III) Total RNA Purification

- ✓ Perform all steps at room temperature and centrifugation $\geq 2,500 \times g$ for 5 minutes (spin-plate).
 - ✓ Do not use the **96-Well Plate Cover Foil** on the spin-plate during RNA Purification.
1. Add 1 volume¹ ethanol (95-100%) to 1 volume sample lysed in **RNA Lysis Buffer** (1:1) and mix well.
 2. Transfer the mixture to a well of the **Silicon A™ Plate**² mounted on a **Collection Plate** and centrifuge³. Discard the flow-through.
 3. **DNase I**⁴ treatment (recommended)
 - (D1) Add 400 μ l **RNA Wash Buffer** and centrifuge. Discard the flow-through.
 - (D2) For each sample/well to be treated, add 5 μ l **DNase I** (1 U/ μ l)*, 35 μ l **DNA Digestion Buffer** and mix by gentle inversion in an RNase-free tube (not included). Add 40 μ l directly to the column matrix of each well.
 - (D3) Incubate at room temperature (20-30°C) for 15 minutes. Proceed to step 4.
 4. Add 400 μ l/well **RNA Prep Buffer** to the plate and centrifuge. Discard the flow-through.
 5. Add 500 μ l/well **RNA Wash Buffer** to the plate and centrifuge. Discard the flow-through and repeat this step.
 6. Mount the plate onto an **Elution Plate** and add 25 μ l **DNase/RNase-Free Water** directly to the matrix and centrifuge.

The eluted RNA⁵ can be used immediately or stored frozen.

Use the **96-Well Cover Foil** to prevent the eluate from evaporation.

1 To isolate large RNA species ≥ 200 nt, add 0.5 volume ethanol (95-100%) to the flow-through and mix well.

2 To process samples $> 600 \mu$ l, plate may be reloaded.

3 At this point, proteins can be purified from the flow-through (page 8).

4 Prior to use, reconstitute the lyophilized **DNase I** (Buffer Preparation, page 4). * Unit definition – one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A_{260} units/ml of reaction mixture at 25°C.

5 For complete removal of PCR (RT) inhibitors from plant, soil and fecal samples, use the OneStep™ PCR Inhibitor Removal Kit (D6030).

Appendices

Samples stabilized and stored in DNA/RNA Shield™

Recommended: **DNA/RNA Shield™** effectively lyses cells, inactivates nucleases and infectious agents and is ideal for sample storage/transport at ambient temperatures prior to nucleic acid purification.

Liquid samples: Mix an equal volume **DNA/RNA Shield™** (2X concentrate) and sample (1:1).

Solid samples: Submerge sample (not to exceed 10% (v/v or w/v) in **DNA/RNA Shield™** (1X).

Mix well/homogenize sample prior to storage. Samples in **DNA/RNA Shield™** can be stored at ambient temperature \geq month or long term at frozen temperature.

Samples in RNAProtect, All Protect, RNAlater, UTM/VTM, saline or PBS

✓ RNAProtect®, All Protect®: Add 3 volumes of **RNA Lysis Buffer** to 1 volume of liquid sample (3:1) and mix well and/or homogenize (e.g., see Tissue, page 5). Proceed to purification, page 6, step 1.

✓ RNAlater™: Add an equal volume of RNase-free water (or PBS) to 1 volume liquid sample (1:1) and mix. Then add 4 volumes **RNA Lysis Buffer** (4:1) to 1 volume sample/water (or PBS). Mix again and proceed to purification, page 6, step 1.

Alternatively, remove the RNAlater™, then proceed with Sample Preparation according to the sample type.

✓ Swab samples in UTM®/VTM®, saline or PBS: Remove swab and add 3 volumes of **RNA Lysis Buffer** to 1 volume sample (3:1). Mix well and proceed to purification, page 6, step 1.

Optional: To inactivate, store and preserve at room temperature prior to purification, add an equal volume of DNA/RNA Shield™ (2X concentrate) to 1 volume of liquid sample (1:1) and mix well. Then proceed to Sample Preparation, Samples in DNA/RNA Shield™, page 5.

Liquids/Reaction Clean-up (DNase I treated RNA, in vitro transcriptions, etc.)

Add 150 μ l **RNA Lysis Buffer** to a \geq 50 μ l liquid sample (3:1) and mix well. Proceed to purification, page 6, step 1.

(Appendices continued)

Protein Purification: Acetone Precipitation of Proteins

- ✓ After the RNA binding, (page 6, step 2), the protein content in the flow-through can be purified:
1. Add 4 volumes of cold acetone (-20°C) to the flow-through (4:1) and mix.
 2. Incubate the samples for 30 minutes on ice.
 3. Centrifuge at max speed for 10 minutes. Discard the supernatant. Keep the pellet.
 4. Add 400 µl ethanol (95-100%) to the protein pellet. Centrifuge at max speed for 1 minute. Discard the supernatant.
 5. Air-dry the protein pellet for 10 minutes at room temperature.
 6. Resuspend and vortex the pellet in a buffer appropriate for downstream application (e.g., SDS-PAGE sample loading buffer).

Input Capacity and Average Total RNA Yield

Input	Average RNA Yield	Kit Capacity
Cells	1 µg (per 10 ⁵ cells)	Up to 10 ⁶
HeLa	1.5 µg	
High Yield Tissue ^{1 (mouse)}	≥ 3 µg (per 10 mg)	Up to 2 mg
Spleen	3-5 µg	
Liver	4-6 µg	
Low Yield Tissue ^{1 (mouse)}	≤ 3 µg (per 1 mg)	Up to 5 mg
Brain, Heart	0.5-1.5 µg	
Muscle	0.5-2 µg	
Lung	1-2 µg	
Intestine	1-3 µg	
Kidney	2-3 µg	
Whole Blood ²	(per 100 µl)	Up to 200 µl
Porcine	1-2 µg	
Human	0.2-1 µg	

1 Yield from tissue can vary due to other factors (i.e., organism type, physiological state and growth conditions).

2 Yield from blood can vary based upon collection, sample preparation, donor, age, and/or health conditions.

(Appendices continued)

Proteinase K Treatment

- ✓ Proteinase K treatment can be performed on protein-rich samples stored in **DNA/RNA Shield™** (2X concentrate; #R1200) (e.g., tissue, blood cells, plasma, serum, saliva, sputum, etc.) using **Proteinase K Set** (#D3001-2-20) and **PK Digestion Buffer** (#R1200-1-20), materials sold separately.
1. For each sample to be treated, prepare **Proteinase K Reaction Mix** in a nuclease-free tube (not included) and mix by vortexing. Scale proportionally, if needed.

Proteinase K Reaction Mix	
Up to 5 mg animal tissue or 10 ⁶ cells in DNA/RNA Shield™	300 µl
PK Digestion Buffer	30 µl
Proteinase K (reconstituted) ³	15 µl

2. Incubate at room temperature (20-30°C) for 30 minutes (homogenized) or 2-5 hours (non-homogenized). Optimization may be required.
3. Add 1 volume **RNA Lysis Buffer** to the treated sample (1:1) and mix. To remove particulate debris, centrifuge and transfer the supernatant into a new nuclease-free tube (not provided). Proceed to purification, page 6.

¹ Reconstitute lyophilized **Proteinase K** (#D3001-2-20; 20 mg) with 1,040 µl **Proteinase K Storage Buffer** and mix by vortexing. Store frozen aliquots.

Ordering Information

Product Description	Catalog No.	Size
Quick-RNA™ 96 Kit	R1052 R1053	2 x 96 preps. 4 x 96 preps.
Individual Kit Components	Catalog No.	Amount
RNA Lysis Buffer	R1060-1-50 R1060-1-100	50 ml 100 ml
RNA Prep Buffer	R1060-2-25 R1060-2-100	25 ml 100 ml
RNA Wash Buffer (concentrate)	R1003-3-24 R1003-3-48	24 ml 48 ml
DNase/RNase-Free Water	W1001-10 W1001-30	10 ml 30 ml
DNase I Set (lyophilized) (250 U supplied with DNA Digestion Buffer, 4 ml)	E1010	1 set
Silicon A™ Plates	C2001	2
Collection Plates	C2002	2
Elution Plates	C2003	2
96-Well Plate Cover Foil	C2007-2 C2007-4	2 pack 4 pack

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	$\leq 10^7$ cells, ≤ 50 mg tissue
MagBeads #R2132/R2133	Automatable (Tecan, Hamilton, Kingfisher, etc.)

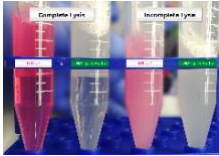
- ✓ For clean-up (purification) and concentration of any RNA sample. (e.g., from the aqueous phase of TRIzol[®] extractions) or from any enzymatic reaction (e.g., DNase I treated RNA):

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
<p>Precipitation, viscous lysate</p>	<p>Incomplete lysis and/or high-mass input:</p> <ul style="list-style-type: none"> - If precipitation occurs (upon adding ethanol to the lysate) or if the lysate is extremely viscous, increase the volume of DNA/RNA Shield™ and/or RNA Lysis Buffer to ensure complete lysis and homogenization until lysate is transparent (see image). 
<p>Low purity (A_{260}/A_{230} nm, A_{260}/A_{280} nm)</p>	<p>Sample handling:</p> <ul style="list-style-type: none"> - 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. <p>Incomplete lysis and/or cellular debris:</p> <ul style="list-style-type: none"> - Increase the volume DNA/RNA Shield™ and/or RNA Lysis Buffer (proportionally) to ensure complete lysis and homogenization. Be sure to centrifuge any cellular debris and then process the cleared lysate.
<p>Low yield</p>	<p>Sample input:</p> <ul style="list-style-type: none"> - 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 DNA/RNA Shield™ and/or RNA Lysis Buffer. <p>High-protein content (blood, plasma/serum, etc.)</p> <ul style="list-style-type: none"> - Perform Proteinase K treatment to the sample prior to purification. See appropriate sample preparation protocol.
<p>DNA contamination</p>	<p>To remove DNA:</p> <ul style="list-style-type: none"> - Perform in-column DNase I treatment (page 6) or perform DNase I treatment post-purification, then re-purify the treated sample. - For future preps, increase the volume of DNA/RNA Shield™ and/or RNA Lysis Buffer to ensure complete lysis and homogenization of the sample.
<p>RNA degradation</p>	<p>To prevent RNA degradation:</p> <ul style="list-style-type: none"> - Immediately collect and lyse fresh sample into DNA/RNA Shield™ and/or RNA Lysis Buffer ensure stability. Homogenized samples can be stored frozen for later processing.

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



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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.

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.

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