



ZYMO RESEARCH

RNA
Purification
Made Simple

Quick-RNA™ Miniprep Kit

RNA from any sample

Highlights

- Spin-column purification of total RNA (including small/microRNAs) from cells and tissue.
- You can opt to isolate total RNA (≥ 17 nt) or isolate small (17-200 nt) and large RNAs (> 200 nt) into separate fractions.
- DNA-free RNA is ready for Next-Gen Sequencing, RT/qPCR, etc. *DNase I is included.*

Catalog Numbers:

R1054, R1055



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



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Table of Contents

Product Contents	01
Specifications	02
Product Description	03
Protocol	04
(I) Buffer Preparation.....	04
(II) Sample Preparation	05
DNA/RNA Shield Samples, Cells, Tissue	05
(III) Total RNA Purification	06
Appendices	07
DNA/RNA Shield Stabilization and Storage	07
RNA Protect, All Protect, RNAlater, UTM/VTM, etc.	07
Liquids/Reaction Clean-up	07
Protein Purification	08
Purify Small and Large RNAs in Separate Fractions	08
Input Capacity and Average Total RNA Yield	09
Proteinase K Treatment	09
Ordering Information	10
Complete Your Workflow	11
Troubleshooting Guide	12
Notes	13
Guarantee	17

Product Contents

Quick-RNA™ Miniprep Kit	R1054 (50 prep)	R1055 (200 prep)
RNA Lysis Buffer	50 ml	100 ml (x2)
RNA Prep Buffer	25 ml	100 ml
RNA Wash Buffer ¹ (concentrate)	24 ml	48 ml (x2)
DNase/RNase-Free Water	6 ml	30 ml
DNase I ² (lyophilized)	250 U	250 U (x4)
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

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

Before use:

1 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).

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

#E1009-A-S (50 U), add 55 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** – **Zymo-Spin™ IIICG Column (green)** yield up to 100 μg RNA.
- **Compatibility** – For samples stored in preservation reagents: **DNA/RNA Shield™**, RNAprotect®, Allprotect®, Universal transport medium/viral transport medium (UTM®/VTM®) and RNeasy Lysis Buffer™.
- **Elution Volume** – ≥ 50 μl **DNase/RNase-Free Water**.
- **Equipment Needed (user provided)** – Microcentrifuge, vortex.

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

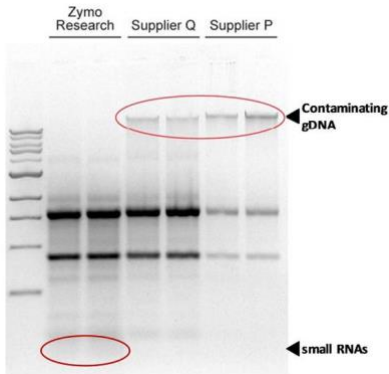
² For urine, DNA/RNA can be isolated with the Quick-DNA/RNA MagBead Kit (R2130, R2131).

Product Description

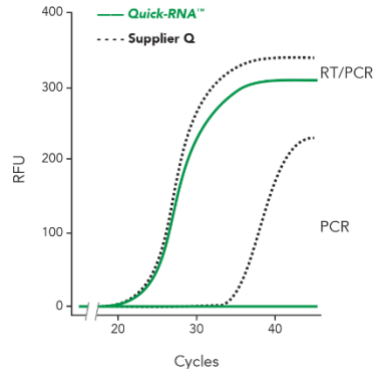
The **Quick-RNA™ Miniprep Kit** provides a quick method for the isolation of high-quality total RNA (≥ 17 nt) from cells (animal, buccal, buffy coat, gram(-) bacteria) and soft, easy-to-lyse tissue. Enrichment of small RNAs (e.g., 17-200 nt; tRNAs, microRNAs) and/or large RNAs (> 200 nt) can be recovered into two separate fractions.

The procedure uses unique spin-column 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.

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

Protocol

The protocol consists of: (I) Buffer Preparation, (II) Sample Preparation and (III) Total RNA Purification.

(I) Buffer Preparation

- ✓ 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 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
#E1009-A-S (50 U), add 55 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
$\leq 5 \times 10^6$	$\leq 10^8$	$\geq 300 \mu\text{l}$
$5 \times 10^6 - 10^7$	$\leq 5 \times 10^8$	$\geq 600 \mu\text{l}$

Proceed to purification, page 6.

Tissue¹

≤ 50 mg low yield tissue (or ≤ 25 mg high yield tissue) can be mechanically homogenized in $\geq 600 \mu\text{l}$ **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 at 10,000-16,000 x g for 30 seconds, unless specified.
- 1. Transfer the sample lysed in **RNA Lysis Buffer** into a **Spin-Away™ Filter¹** (**yellow**) in a **Collection Tube** and centrifuge to remove the majority of genomic DNA.

Save the flow-through!

2. Add 1 volume² ethanol (95-100%) to the flow-through (1:1) and mix well. Then transfer the sample into a **Zymo-Spin™ IIICG Column¹** (**green**) in a **Collection Tube** and centrifuge³. Discard the flow-through.
3. **DNase I⁴** treatment (recommended)
 - (D1) Wash the column with 400 µl **RNA Wash Buffer** and centrifuge. Discard the flow-through.
 - (D2) In an nuclease-free tube, add 5 µl **DNase I** (1 U/µl)*, 75 µl **DNA Digestion Buffer** and mix. Add mixture directly into the column matrix.
 - (D3) Incubate the column at room temperature (20-30°C) for 15 minutes.
4. Add 400 µl **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
5. Add 700 µl **RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.
6. Add 400 µl **RNA Wash Buffer** and centrifuge the column for 1 minute to ensure complete removal of the wash buffer. Then carefully, transfer the column into a nuclease-free tube (not provided).
7. Add 100 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

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

The eluted RNA⁵ can be used immediately or stored frozen.

1 To process samples > 700 µl, columns may be reloaded.

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

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 A₂₆₀ 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 2.

✓ RNAlater™: Add 1 volume of RNase-free water (or PBS) to 1 volume liquid sample (1:1) and mix. Then add 4 volumes **RNA Lysis Buffer** to 1 volume sample/water (or PBS). Mix again and proceed to purification, page 6, step 2.

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 2.

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 2.

(Appendices continued)

Protein Purification: Acetone Precipitation of Proteins

- ✓ After the RNA binding to the column (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).

Purification of Small and Large RNAs into Separate Fractions

- ✓ This procedure is compatible with animal cell inputs ($\leq 10^6$) or purified RNA only.
- ✓ Perform all steps at room temperature and centrifugation steps at 10,000-16,000 x g for 30 seconds, unless specified.

1. Prepare adjusted **RNA Lysis Buffer** (as needed). Mix an equal volume of buffer and ethanol (95-100%).

Example: Mix 50 µl buffer and 50 µl ethanol.

2. Add 2 volumes of the adjusted buffer to the sample¹ and mix.

Example: Mix 100 µl adjusted buffer and 50 µl sample.

3. Transfer the mixture to the **Zymo-Spin™ Column**² and centrifuge. **Save the flow-through!**

4. **Small RNAs (17-200 nt) are in the flow-through**

- a. Add 1 volume ethanol and mix.

Example: Add 150 µl ethanol to 150 µl sample.

- b. Transfer the mixture to a **new column** and centrifuge. Discard the flow-through.
- c. Proceed with purification, page 6, step 4.

4. **Large RNAs (> 200 nt) are retained in the column**

- a. Proceed with purification, page 6, step 4.

¹ To minimize pipetting error, adjust the sample volume to 50 µl (minimum).

² To process samples > 700 µl, columns may be reloaded.

(Appendices continued)

Input Capacity and Average Total RNA Yield

Input	Average RNA Yield	Kit Capacity
Cells	10 µg (per 10 ⁶ cells)	Up to 10 ⁷
HeLa	15 µg	
High Yield Tissue ^{1 (mouse)}	≥ 30 µg (per 10 mg)	Up to 20 mg
Spleen	30-50 µg	
Liver	40-60 µg	
Low Yield Tissue ^{1 (mouse)}	≤ 30 µg (per 10 mg)	Up to 50 mg
Brain, Heart	5-15 µg	
Muscle	5-20 µg	
Lung	10-20 µg	
Intestine	10-30 µg	
Kidney	20-30 µg	
Whole Blood ²	(per 1 ml)	Up to 3 ml
Porcine	10-20 µg	
Human	2-10 µg	

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 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.

3 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™ Miniprep Kit	R1054 R1055	50 preps. 200 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
Spin-Away™ Filters	C1006-50-F	50
Zymo-Spin™ IICG Columns	C1006-50-G	50
Collection Tubes	C1001-50	50

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	
Microprep #R1050	From 1 cell and up
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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