ZYMO RESEARCH

RNA Purification Made Simple™

Quick-RNA™ Microprep Kit

RNA from any sample

Highlights

- Spin-column purification of total RNA (including small/microRNAs) from cells and tissue.
- Extract total RNA from low inputs (down to a single cell).
- 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:
R1050, R1051

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

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682

Table of Contents

Product Contents

Quick-RNA™ Microprep Kit	R1050 (50 prep)	R1051 (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	4 ml	10 ml
DNase I[2] (lyophilized)	250 U	250 U (x4)
DNA Digestion Buffer	4 ml	16 ml
Zymo-Spin™ IC Columns	50	200
Collection Tubes	50	200
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 (R1050) or 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate (R1051).
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, gram(-) bacteria), soft and easy-to-lyse tissue, samples in **DNA/RNA Shield™** or other preservation reagents, and enzymatic reactions (e.g., DNase I treated, Proteinase K treated). Not compatible with whole blood[1] and urine[1] 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 7).

- **Binding Capacity – Zymo-Spin™ IC Column** yield up to 10 µg RNA.

- **Compatibility –** For samples stored in preservation reagents: **DNA/RNA Shield™**, RNAprotect®, Allprotect®, Universal transport medium/viral transport medium (UTM®/VTM®) and RNAlater™.

- **Elution Volume –** ≥ 6 µl **DNase/RNase-Free Water.**

- **Equipment Needed (user provided) –** Microcentrifuge, vortex.

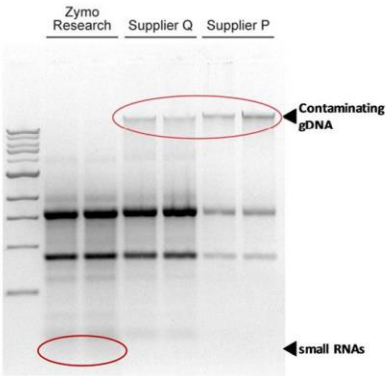
1 For RNA purification from whole-blood and urine, see the Quick-RNA Miniprep Plus Kit (R1057, R1058) or the Quick-RNA MagBead Kit (R2132, R2133).

Product Description

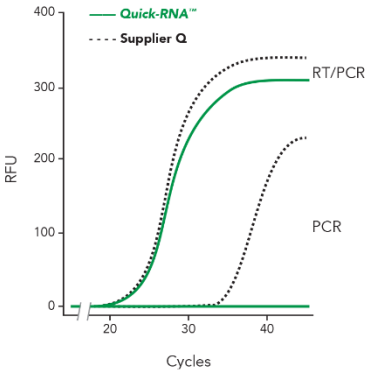
The **Quick-RNA™ Microprep Kit** provides a quick method for the isolation of high-quality total RNA (≥ 17 nt) from a low number (1-10^6) of cells (animal, buccal, buffy coat, gram(-) bacteria) and soft, easy-to-lyse tissue. Enrichment of small RNAs (e.g., 17-200 nt; tRNAs, microRNAs) and/or large RNAs (> 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.

Input Capacity and 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[1,2 (mouse)]	≥ 3 µg (per 1 mg)	Up to 2 mg
Spleen	3-5 µg	
Liver	4-6 µg	
Low Yield Tissue[1,2 (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[1,3]	(per 100 µl)	Up to 200 µl
Porcine	1-2 µg	
Human	0.2-1 µg	

1 Recommended: For protein-rich samples (e.g., tissue, blood cells, etc.) stored in DNA/RNA Shield™ (#R1100, sold separately), Proteinase K treatment can be performed using Proteinase K Set (#D3001-2-5, #D2001-2-20; sold separately) and PK Digestion Buffer (#R1200-1-5, #R1200-1-20; sold separately), see page 9.
2 Yield from tissue can vary due to other factors (i.e., organism type, physiological state, and growth conditions.
3 Yield from blood can vary based upon collection, sample preparation, donor, age, and/or health conditions.

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 (R1050) or 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate (R1051).

- ✓ 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**
  #E1011-A (1500 U), add 1,500 µl **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, etc.)

1. If frozen, thaw homogenized sample in **DNA/RNA Shield™** to room temperature (20-30°C). Mix well by vortex.
2. Add an equal volume of **RNA Lysis Buffer** (1:1), mix well and proceed to purification, page 7.

Cells & Tissue (mammalian)

1. **Cells**:
   a. If in suspension[1], pellet by centrifugation (≤ 500 x g for 1 minute), remove supernatant and resuspend cell pellet in **RNA Lysis Buffer** (see table below).
   b. If adherent, 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, etc.

Cells	Gram(-) Bacteria	Add RNA Lysis Buffer
≤ 10^5	-	≥ 100 µl
≤ 10^6	≤ 10^8	≥ 300 µl

To remove particulate debris, centrifuge and transfer the supernatant into a nuclease-free tube (not provided). Proceed to purification, page 7.

2. **Tissue**[2]:
   a. Submerge an appropriate amount of fresh or frozen sample (see table below) into **RNA Lysis Buffer** and homogenize[3,4].

Tissue	Add RNA Lysis Buffer
High-yield (≤ 2 mg) Low-yield (≤ 5 mg)	≤ 600 µl

   b. To remove particulate debris, centrifuge and transfer the cleared supernatant into a nuclease-free tube (not provided). Proceed to purification, page 7.

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1 If liquid/media cannot be removed, add ≥ 3 volumes **RNA Lysis Buffer** to 1 volume liquid sample (3:1) and mix well. Proceed to purification, page 7.
2 For examples of sample type input and average yield, see chart on page 4.
3 For efficient homogenization, bead beat samples with ZR BashingBead Lysis Tubes (S6012, S6003), sold separately. See Appendices (page 9) for bead beating parameters. Other types of homogenization can include mortar/pestle, dounce, syringe or tissue grinder, etc.
4 If no method of homogenization, alternatively tissue samples collected and stored in DNA/RNA Shield™ can be Proteinase K treated. See Appendices (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. Add 1 volume[1] ethanol (95-100%) to 1 volume sample lysed in **RNA Lysis Buffer** (1:1) and mix well.

   Example: Add 300 µl ethanol to 300 µl mixture (sample lysed in **RNA Lysis Buffer**).

2. Transfer the mixture into a **Zymo-Spin™ IC Column**[2] in a **Collection Tube** and centrifuge[3]. Discard the flow-through.

3. **DNase I**[4] 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)*, 35 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 15 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

   Alternatively, for highly concentrated RNA use ≥ 6 µl elution.

   The eluted RNA[5] can be used immediately or stored frozen.

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1 To isolate large RNA species ≥ 200 nt, add 0.5 volume ethanol (95-100%) to 1 volume sample lysed in RNA Lysis Buffer (0.5:1) and mix well.
2 To process samples > 700 µl, columns may be reloaded.
3 At this point, proteins can be purified from the flow-through (page 10).
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

Sample stabilization and storage in DNA/RNA Shield™

Liquid samples (e.g., whole-blood): Add 3 volumes **DNA/RNA Shield™** (1X) to 1 volume sample (3:1). Mix well.

Solid samples (e.g., pelleted cells, tissue): Submerge sample (not to exceed 10% (v/v or w/v)) in **DNA/RNA Shield™** (1X) and homogenize (see Appendices, page 9).

Store samples in **DNA/RNA Shield™** at ambient temperature for ≥ 1 month or long term at frozen temperature. **DNA/RNA Shield™** is directly compatible with most guanidinium-based extraction methods (e.g., no need to remove reagent from the lysed/homogenized sample).

Samples in RNAprotect, Allprotect, RNAlater, UTM/VTM, saline or PBS

- ✓ RNAProtect®, Allprotect®: Add 3 volumes of **RNA Lysis Buffer** to 1 volume of liquid sample (3:1). Mix well and/or homogenize base on sample type (see Sample Preparation, page 6). Then, proceed to purification, page 7.
- ✓ RNAlater™:
  a. Cells - Pellet[1] by centrifugation at up to 5,000 x g and remove RNAlater (supernatant). Proceed to Sample Preparation, page 6.
  b. Tissue - Transfer into a new tube with forceps and remove any excess RNAlater™. Proceed to Sample Preparation, page 6.

  Alternatively, for liquid samples from which RNAlater cannot be removed, add 1 volume of nuclease-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) mixture (4:1). Mix again and proceed to Total RNA Purification, page 7.
- ✓ 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 7.

  Optional: To inactivate pathogens, store at room temperature prior to purification, add 1 volume **DNA/RNA Shield™** (2X concentrate) to 1 volume liquid sample (1:1) and mix well. Then proceed to Sample Preparation, Samples in **DNA/RNA Shield™**, page 6.

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

Add 150 µl **RNA Lysis Buffer** to a ≥ 50 µl liquid sample (3:1) and mix well. Proceed to purification, page 7, step 1.

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1 Different cells may react differently to centrifugation forces, and it is recommended to test the pelleting procedure with non-valuable samples first. Diluting RNAlater™ by 50% with cold PBS reduces solution density allowing for lower forces during cell pelleting (e.g., 500 x g).

(Appendices continued)

Homogenization with ZR BashingBead Lysis Tubes

- ✓ Recommended for complete and efficient homogenization of tough-to-lyse samples (e.g., tissue, plant, seed, microbes, etc.). Lysis tubes sold separately.
- ✓ For high-speed (e.g., MP Bio FastPrep-24, Bertin Precellys) and low-speed (e.g., Vortex Genie) homogenizers, bead-beating time optimization may be required.

Input	Tissue		Microbes
	Mammalian	Plant/Seed or Insect	Bacteria, Swab, Yeast, Stool/Soil
Cat. no. (lysis bead size)	S6003 (2.0 mm)	S6003 (2.0 mm)	S6012 (0.5 mm and 0.1 mm)
High-speed	30-60 sec	3-5 min	30-60 sec
Low-speed	3-5 min	15-20 min	5-10 min

Proteinase K Treatment

- ✓ Proteinase K treatment (optional) can be performed on protein-rich samples stored in **DNA/RNA Shield™** (#R1100, sold separately) (e.g., tissue, blood cells, etc.), using **Proteinase K Set** (#D3001-2-5, #D2001-2-20; sold separately) and **PK Digestion Buffer** (#R1200-1-5, #R1200-1-20; sold separately).

1. For every 300 µl of **DNA/RNA Shield™** sample, add 15 µl **Proteinase K** and 30 µl **PK Digestion Buffer**. Mix and incubate at room temperature (20-30°C) for ≥30 minutes (homogenized) or 2-5 hours (non-homogenized). Optimization may be required.
2. To remove particulate debris from homogenized tissue, centrifuge and transfer the cleared supernatant into a nuclease-free tube (not provided).
3. Add **RNA Lysis Buffer** to the supernatant (1:1) and mix well. Proceed to purification, page 7.

1 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.

(Appendices continued)

Protein Purification: Acetone Precipitation of Proteins

✓ After the RNA binding to the column (page 7, step 2), protein content (denatured) in the flow-through can be purified:

1. Add 4 volumes of cold acetone (-20°C) to 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 (≤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) by mixing an equal volume of buffer and ethanol (95-100%) (1:1).

   Example: Mix 50 µl buffer and 50 µl ethanol.

2. Add 2 volumes of the adjusted buffer to the sample[1] and mix.

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

3. Transfer the mixture to the **Zymo-Spin™ IC 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 7, step 4.

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

   a. Proceed with purification, page 7, 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.

Ordering Information

Product Description	Catalog No.	Size
Quick-RNA™ Microprep Kit	R1050 R1051	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
Zymo-Spin™ IC Columns	C1004-50	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 DNA/RNA from any sample:

Quick-DNA/RNA Plus kits	
Microprep Plus #D7005	From 1 cell and up
MagBeads #R2130	Automatable (Tecan, Hamilton, Kingfisher, etc.)

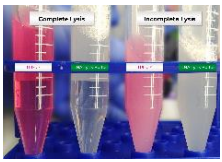
✓ For clean-up (purification) and concentration of any RNA sample. (e.g., from the aqueous phase of TRIzol® extractions) or from any enzymatic reaction (e.g., DNase I treated RNA):

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
Precipitation, viscous lysate	Incomplete lysis and/or high-mass input: - 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). 
Low purity (A_{260}/A_{230} nm, A_{260}/A_{280} nm)	Sample handling: - 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. Incomplete lysis and/or cellular debris: - Increase the volume DNA/RNA Shield™ and/or RNA Lysis Buffer (proportionally) to ensure complete lysis and homogenization. Be sure to centrifuge and pellet any cellular debris then process the cleared lysate.
Low yield	Sample input: - 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. High-protein content (blood, plasma/serum, etc.) - Perform Proteinase K treatment to the sample prior to purification. See Appendices.
DNA contamination	To remove DNA: - Perform in-column DNase I treatment (page 9) 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.
RNA degradation	To prevent RNA degradation: - 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

Notes

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