ZYMO RESEARCH

RNA Purification Made Simple™

Quick-RNA™ Miniprep Plus Kit

RNA from any sample

Highlights

- Spin-column purification of total RNA (including small/microRNAs) from any sample including cells, solid tissue, biological liquids, environmental samples, swabs, and any sample in DNA/RNA Shield™
- DNA/RNA Shield™ and Proteinase K are included for unique preservation and lysis technology.
- DNA-free RNA is ready for Next-Gen Sequencing, RT/qPCR, etc. *DNase I is included.*

Catalog Numbers:
R1057T, R1057, R1058

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

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Table of Contents

Product Contents

Quick-RNA™ Miniprep Plus Kit	R1057T (10 prep)	R1057 (50 prep)	R1058 (200 prep)
RNA Lysis Buffer	10 ml	50 ml	100 ml (x2)
RNA Prep Buffer	5 ml	25 ml	100 ml
RNA Wash Buffer[1]	16 ml (ready-to-use)	24 ml (concentrate)	48 ml (x2)
DNase/RNase-Free Water	1 ml	6 ml	30 ml
DNase I[2] (lyophilized)	50 U	250 U	250 U (x4)
DNA Digestion Buffer	0.8 ml	4 ml	16 ml
DNA/RNA Shield™ (2X concentrate)	5 ml	25 ml	125 ml
PK Digestion Buffer	1 ml	5 ml	20 ml
Proteinase K[3] (lyophilized) & Storage Buffer	5 mg (x2)	60 mg	60 mg (x3)
Spin-Away™ Filters	10	50	200
Zymo-Spin™ IIICG Columns	10	50	200
Collection Tubes	20	100	400
Instruction Manual	1 pc	1 pc	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 (R1057) or 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate (R1058). **RNA Wash Buffer** (R1057T) is supplied ready-to-use and does not require the addition of ethanol.
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**
3 Add **Proteinase K Storage Buffer** to the lyophilized **Proteinase K**, 20 mg, see Buffer Preparation, page 5. Store frozen aliquots.

Specifications

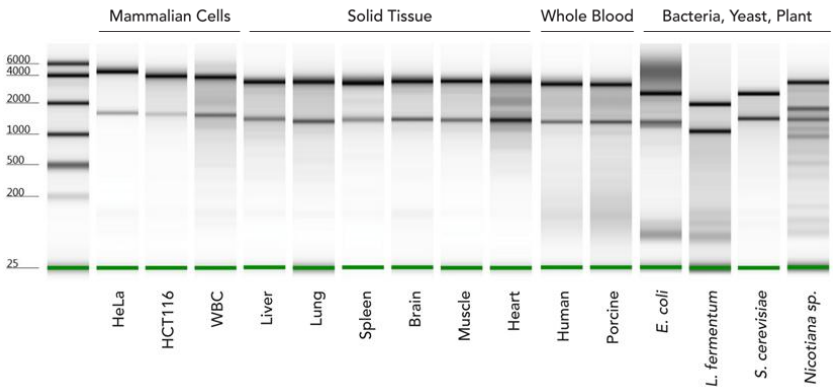
- **Sample Sources –** Any cells (animal, bacterial, blood cells, etc.), all tissues (tough-to-lyse, FFPE, etc.), blood, biological fluids, enzymatic reactions (e.g., DNase I treated) and samples in **DNA/RNA Shield™** or other preservation reagents.
- **Sample Preservation and Inactivation – DNA/RNA Shield™** lyses cells, inactivates nucleases and infectious agents (e.g., virus, pathogens) and is ideal for safe sample storage and transport at ambient temperatures (page 11).
- **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 10).
- **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®), PAXgene® and RNAlater™.
- **Elution Volume –** ≥ 50 µl **DNase/RNase-Free Water.**
- **Equipment Needed** (user provided) **–** Microcentrifuge, vortex, heat block, water bath or incubator.

Product Description

The **Quick-RNA™ Miniprep Plus Kit** combines **Quick-RNA™** technology with the addition of **DNA/RNA Shield™**, a unique preservation and lysis technology, and **Proteinase K** to enable easy, reliable, and rapid isolation of RNA from any biological sample including any cells, all tissues, blood, and other biological fluids.

The procedure uses unique spin-column technology that results in high-quality total RNA (including small RNAs 17-200 nt) and is ready for Next-Gen Sequencing, RT/qPCR, hybridization, etc.

High-Quality RNA From Any Sample Type



High quality total RNA is isolated from various sample types including mammalian cells, solid tissue, whole blood, bacteria, yeast, and plant using the ***Quick*-RNA™ Plus** kits (Agilent 2200 TapeStation™).

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	

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.

Protocol

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

(I) Buffer Preparation

- ✓ Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate (R1057) or 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate (R1058). **RNA Wash Buffer** (R1057T) is supplied ready-to-use and does not require the addition of ethanol.

- ✓ 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**

- ✓ Reconstitute lyophilized **Proteinase K** at 20 mg/ml with **Proteinase K Storage Buffer** and mix by vortexing. Use immediately or store frozen aliquots:
  #D3001-2-60 (60 mg), add 3.12 ml **buffer**
  #D3001-2-5 (5 mg), add 0.26 ml **buffer**

- ✓ To prepare a 1X solution, add an equal volume of nuclease-free water (not provided) to the **DNA/RNA Shield™** (2X concentrate) (1:1) and mix well.

(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. Proceed to the appropriate procedure below based on sample type (omit the step involving the addition of **DNA/RNA Shield™**).

Cells & Tissue (mammalian)

1. For samples (cells or tissue) already stored in **DNA/RNA Shield™**, add an equal volume of **RNA Lysis Buffer** (1:1), mix well and proceed to purification, page 10.
2. **Cells**: Pellet cell suspension by centrifugation (≤ 500 x g for 1 minute) or process adherent cells directly in culture container. Remove supernatant/media[1] respectively and resuspend cells in **RNA Lysis Buffer** (see table below).

Cells	Add RNA Lysis Buffer
≤ $5x10^6$	≥ 300 µl
$5x10^6$ - 10^7	≥ 600 µl

   Remove particulate debris by centrifugation and transfer the supernatant into a nuclease-free tube (not provided). Proceed to purification, page 10.

3. **Tissue**[2]: Submerge an appropriate amount of fresh or frozen sample (see table below) into **DNA/RNA Shield™** (1X)[3] and homogenize[4,5].

Tissue	Add DNA/RNA Shield (1X)
High-yield (≤ 25 mg) Low-yield (≤ 50 mg)	≤ 600 µl

   a. For every 300 µl of 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.
   b. To remove particulate debris, centrifuge and transfer the cleared supernatant into a nuclease-free tube (not provided).
   c. Add an equal volume of **RNA Lysis Buffer** to the supernatant (1:1) and mix well. Proceed to purification, page 10.

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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 10.
2 For examples of sample type input and average yield, see chart on page 4.
3 For a 1X solution of **DNA/RNA Shield™,** see Buffer Preparation, page 5.
4 For efficient homogenization, bead beat samples with ZR BashingBead Lysis Tubes (S6012, S6003), sold separately. See Appendices (page 12) for bead beating parameters. Other types of homogenization can include mortar/pestle, dounce, syringe or tissue grinder, etc.
5 Alternatively (if no homogenization), tissue samples can be Proteinase K treated only (proceed to step 3a).

Tough-to-Lyse Samples (bacteria, yeast, insect, swab, soil[1], stool[1], plant[1], seed[1])

1. Add 800 µl of **DNA/RNA Shield**™ (1X)[2] to an appropriate amount of sample (see table below) and homogenize[3] (e.g., bead beating).

Solid Tissue	Microbes	Add DNA/RNA Shield (1X)
Plant/Seed or Insect (≤ 200 mg)	Bacteria (≤ 10^9) Yeast (≤ 10^8) Swab, Stool/Soil (≤ 50 mg)	800 µl

2. After homogenization, remove particulate debris by centrifugation at max speed. Transfer the cleared supernatant into a nuclease-free tube (not provided).
3. Add **RNA Lysis Buffer** to the supernatant (1:1), mix well and proceed with purification, page 10.

FFPE Tissue

1. Remove (trim) excess paraffin wax from ≤ 25 mg FFPE tissue and transfer into a nuclease-free tube (not provided).
2. Add 400 µl **Deparaffinization Solution**[4] to the sample. Incubate at 55ºC for 1 minute. Vortex briefly. Remove the **Deparaffinization Solution**.
3. Add 95 µl **DNase/RNase-Free Water**, 95 µl **2X Digestion Buffer**[4], and 10 µl **Proteinase K**. Mix well.
4. Incubate at 55ºC for 1 hour. Then incubate at 65ºC for 15 minutes to de-crosslink the sample.
5. Centrifuge to remove insoluble debris and transfer 200 µl supernatant to a nuclease-free tube (not provided).
6. Add **RNA Lysis Buffer** to the supernatant (1:1) and mix well. Proceed to purification, page 10.

Blood Cells (mammalian, PBMCs, WBCs, etc.)

1. For blood cells, buffy coat and pelleted PAXgene® or RNAlater™ samples, resuspend in **DNA/RNA Shield**™ (1X)[2].

Blood Cells	Add DNA/RNA Shield™ (1X)
≤ 5 ml blood (≤ 10^7 cells)	300 µl

2. For every 300 µl of sample, add 15 µl **Proteinase K** and 30 µl **PK Digestion Buffer**. Continue to step 3, page 8.

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1 For PCR inhibitor removal, use OneStep PCR Inhibitor Removal Kit (D6030).
2 For a 1X solution of **DNA/RNA Shield**™, see Buffer Preparation, page 5.
3 For efficient homogenization, bead beat samples with ZR BashingBead Lysis Tubes (S6012, S6003), sold separately. See Appendices (page 12) for bead beating parameters.
4 Deparaffinization Solution (D3067-1-20) and 2X Digestion Buffer (D3050-1-20) are sold separately.

3. Mix and incubate at room temperature (20-30°C) for ≥ 30 minutes. Optimization may be required.
4. After incubation, vortex sample and centrifuge at max speed for 2 minutes to pellet debris. Transfer 300 µl of the cleared supernatant to a nuclease-free tube (not provided).
5. Add **RNA Lysis Buffer** to the supernatant (1:1) and mix well. Proceed to purification, page 10.

Whole Blood[1,2] (mammalian)

1. Add 200 µl **DNA/RNA Shield™** (2X concentrate) directly to each 200 µl of fresh or frozen blood sample and mix thoroughly[3].
2. For every 400 µl of reagent/blood mixture, add 8 µl **Proteinase K** and mix well. Incubate at room temperature (20-30°C) for 30 minutes.
3. After incubation, vortex sample and centrifuge at max speed for 2 minutes to pellet debris. Transfer the cleared supernatant to a new nuclease-free tube (not provided).
4. Add an equal volume of isopropanol (1:1) and mix well.
5. Transfer the mixture into a **Zymo-Spin™ IIICG Column**[4] (green) in a Collection Tube and centrifuge[4]. Discard the flow-through and proceed to purification, page 10, step 3.

Saliva & Buccal Cells

1. For saliva and buccal cell samples, add an equal volume of **DNA/RNA Shield™** (2X) (1:1).

Saliva & Buccal Cells	Add DNA/RNA Shield™ (2X)
200 µl (≤ 10^7 cells)	200 µl

2. For every 400 µl of reagent/sample mixture, add 20 µl **Proteinase K** and 40 µl **PK Digestion Buffer**.
3. Mix and incubate at room temperature (20-30°C) for ≥ 30 minutes. Optimization may be required.
4. After incubation, vortex sample and centrifuge at max speed for 2 minutes to pellet debris. Transfer 400 µl of the cleared supernatant to a nuclease-free tube (not provided).
5. Add **RNA Lysis Buffer** to the supernatant (1:1) and mix well. Proceed to purification, page 10.

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1 Compatible with commonly used anticoagulants (e.g., EDTA, citrate, heparin)
2 Up to 3 ml blood per prep can be processed (by reloading the column).
3 To retain protein in the whole blood sample, omit step 2 and continue to step 3.
4 To process samples > 700 µl, columns may be reloaded.

Urine[1]

1. Generate pellet from up to 40 ml urine by adding 70 µl **Urine Conditioning Buffer**[2] for every 1 ml of urine and mix by vortex. Centrifuge at 3,000 x g for 15 minutes. Discard the supernatant and keep the pellet. Add **DNA/RNA Shield™** (1X)[3] and mix by pipetting.

Pelleted cells from urine	Add DNA/RNA Shield™ (1X)
≤ 40 ml urine	300 µl

2. For every 300 µl of sample, add 15 µl **Proteinase K**.
3. Mix and incubate at room temperature (20-30°C) for ≥ 30 minutes. Optimization may be required.
4. After incubation, vortex sample and centrifuge at max speed for 2 minutes to pellet debris. Transfer 300 µl of the cleared supernatant to a nuclease-free tube (not provided).
5. Add **RNA Lysis Buffer** to the supernatant (1:1) and mix well. Proceed to purification, page 10.

1 Warm up urine sample at 37°C for 5-10 minutes if there is visual precipitation or cloudiness. Samples that contain bacterial contamination will not be clear.
2 Urine Conditioning Buffer (D3061-1-8, D30601-1-140) is sold separately.
3 For a 1X solution of **DNA/RNA Shield™**, see Buffer Preparation, page 5.

(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™ Filter**[1] (**yellow**) in a **Collection Tube** and centrifuge to remove the majority of genomic DNA.

   Save the flow-through!

2. Add 1 volume[2] ethanol (95-100%) to the flow-through (1:1) and mix well.

   Example: Add 300 µl ethanol to 300 µl flow-through.

   Then transfer the mixture into a **Zymo-Spin™ IIICG Column**[1] (**green**) 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)*, 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 RNA[5] can be used immediately or stored frozen.

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1 To process samples > 700 µl, columns may be reloaded.
2 To isolate only large RNA species ≥ 200 nt, add 0.5 volume ethanol (95-100%) to flow-through and mix well.
3 Optional: At this point, proteins can be purified from the flow-through (page 12).
4 Prior to use, reconstitute the lyophilized **DNase I** (Buffer Preparation, page 5). * 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)[1] to 1 volume sample (3:1). Mix well.

Solid samples (e.g., tissue): Submerge sample (not to exceed 10% (v/v or w/v)) in **DNA/RNA Shield™** (1X)[1] and homogenize (see Appendices, page 12).

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 stored sample prior to extraction).

Samples in RNAprotect, Allprotect, RNAlater, PAXgene, 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 Total RNA Purification, page 10.
- ✓ RNAlater™:
  a. Cells - Pellet[2] 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 10.
- ✓ PAXgene®: Refer to manufacturer's instructions to remove the reagent then proceed to Sample Preparation, Blood Cells, 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 and aliquot every 200 µl of mixture into a nuclease-free tube. Proceed to Total RNA Purification, page 10.

  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.

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1 For a 1X solution of **DNA/RNA Shield™**, see Buffer Preparation, page 5.
2 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)

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 10.

Protein Purification: Acetone Precipitation of Proteins

- ✓ After the RNA binding to the column (page 10, step 2), the 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).

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 homogenizers (e.g., MP Bio FastPrep-24, Bertin Precellys) and low-speed homogenizers (e.g., Vortex Genie), 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

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)

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™ 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 10, step 4.

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

   a. Proceed with purification, page 10, 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™ Miniprep Plus Kit	R1057T R1057 R1058	10 preps. 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
DNA/RNA Shield™ (2X concentrate)	R1200-25 R1200-125	25 ml 125 ml
PK Digestion Buffer	R1200-1-5 R1200-1-20	5 ml 20 ml
Proteinase K (lyophilized) & Storage Buffer	D3001-2-5 D3001-2-60	5 mg 60 mg
Spin-Away™ Filters	C1006-50-F	50
Zymo-Spin™ IIICG 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 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.)

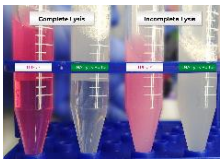
✓ For clean-up (purification) and concentration of any RNA sample. (e.g., from the aqueous phase of TRIzol® extractions) or from any enzymatic reaction (e.g., DNase I treated RNA):

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 appropriate sample preparation protocol.
DNA contamination	To remove DNA: - Perform in-column DNase I treatment (page 10) 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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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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