ZYMO RESEARCH

RNA Purification Made Simple™

Quick-RNA™ MagBead

RNA from any sample

Highlights

- High-throughput, magnetic-bead 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:
R2132, R2133

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

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Table of Contents

Product Contents

Quick-RNA™ MagBead	R2132 (96 prep)	R2133 (4 x 96 prep)
DNA/RNA Shield™ (2X concentrate)	25 ml	125 ml
Proteinase K[1] (lyophilized) & Storage Buffer	20 mg	20 mg (x4)
RNA Lysis Buffer	50 ml	200 ml
MagBinding Beads	6 ml	24 ml
MagBead DNA/RNA Wash 1[2] (concentrate)	30 ml (x2)	120 ml (x2)
MagBead DNA/RNA Wash 2[3] (concentrate)	20 ml (x2)	80 ml (x2)
DNase I[1] (lyophilized)	250 U (x3)	1500 U (x2)
DNA Digestion Buffer	4 ml	4 ml
RNA Prep Buffer	50 ml	100 ml (x2)
DNase/RNase-Free Water	30 ml	100 ml
Instruction Manual	1 pc	1 pc

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature.
Before use:
1 Reconstitute the lyophilized **Proteinase K** and **DNase I** according to Buffer Preparation, page 5.
2 Add 20 ml (R2132) or 80 ml (R2133) of isopropanol to the **MagBead DNA/RNA Wash 1** (concentrate).
3 Add 30 ml (R2132) or 120 ml (R2133) of isopropanol to the **MagBead DNA/RNA Wash 2** (concentrate).

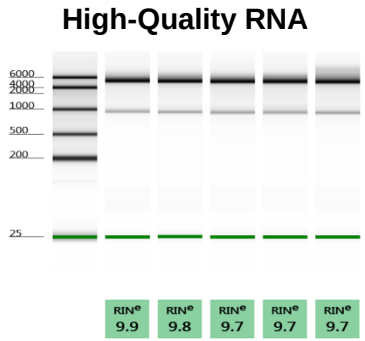
Specifications

- **Sample Sources –** Any cells (animal, bacterial, blood cells, etc.), all tissues (tough-to-lyse, FFPE, etc.), blood, biological fluids, environmental (plant/seed), swabs (stool, soil, microbial samples), 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.
- **Binding Capacity –** 15 µg RNA per 30 µl **MagBinding Beads**.
- **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) **–** Magnetic stand or separator, heat block, liquid handler, or robotic sample processer (user provided) and nuclease-free tubes.
- **Recommended Materials** (sold separately) **–** 96-well Collection Plate (C2002; capacity is up to 1.2 ml/well), 96-Well Block (P1001; capacity is up to 2 ml/well), 96-well Elution Plate (C2003), Cover Foil (C2007), ZR-96 MagStand (P1005), DNase/RNase-Free Tubes (1.5 ml; C2001).

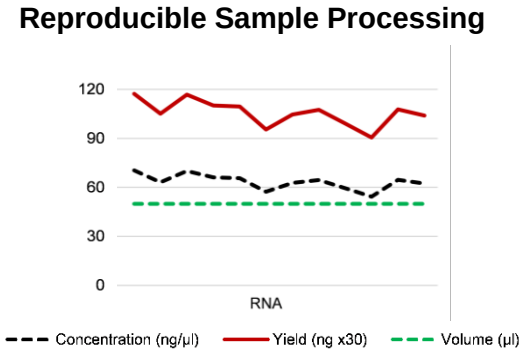
Product Description

The **Quick-RNA™ MagBead** kit provides a high-throughput, magnetic bead-based purification of total RNA (including small/microRNAs). The provided **DNA/RNA Shield™** inactivates infectious agents and is ideal for sample storage at ambient temperatures.

Total RNA is eluted into ≥ 50 µl of **DNase/RNase-Free Water** and is ready for any downstream application including Next-Gen Sequencing, RT/PCR, hybridization, *etc.*



Total RNA quality is assessed using Agilent 2200 TapeStation. RNA were purified from HeLa cells using the ***Quick*-RNA™ MagBead** kit.



Concentration, yield, and elution volume across replicate samples extracted with the ***Quick*-RNA™ MagBead** are reproducible and consistent. RNA were purified from HeLa cells (2.5 x 10^5/well).

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 1 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.

Protocol

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

(I) Buffer Preparation

- ✓ Add 20 ml (R2132) or 80 ml (R2133) isopropanol to the **MagBead DNA/RNA Wash 1** concentrate.
- ✓ Add 30 ml (R2132) or 120 ml (R2133) isopropanol to the **MagBead DNA/RNA Wash 2** concentrate.
- ✓ To prepare 1X solution of **DNA/RNA Shield™**, mix equal amounts of the supplied 2X concentrate with nuclease-free water (not provided) and mix well.
- ✓ Reconstitute lyophilized **Proteinase K** at 20 mg/ml with **Proteinase K Storage Buffer** and mix by vortexing. Use immediately or store frozen aliquots:
  #D3001-2-20 (20 mg), add 1.04 ml **buffer**
- ✓ Reconstitute each vial of lyophilized **DNase I** with **DNase/RNase-Free Water** in a conical tube (not provided). Mix by gentle inversion and store frozen aliquots.
  #E1011-A (1500 U), add 13.5 ml **water**
  #E1009-A (250 U), add 2.25 ml **water**

  For each sample to be treated, prepare **DNase I Reaction Mix** (scale up proportionally): Add 45 µl **DNase I** (reconstituted) and 5 µl **DNA Digestion Buffer** in a nuclease-free tube (not provided), mix by gentle inversion and place on ice until ready to use.

(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™**, transfer 200 µl of sample to a new nuclease-free tube (not provided), and proceed to Total RNA 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 **DNA/RNA Shield™** (1X)[2] (see table below).

Cells	Add DNA/RNA Shield (1X)
≤ 10^6	≥ 200 µl

   Remove debris by centrifugation and transfer every 200 µl of the cleared supernatant into a nuclease-free tube (not provided). Proceed to Total RNA Purification, page 10.

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

Tissue	Add DNA/RNA Shield (1X)
High-yield (≤ 2 mg) Low-yield (≤ 5 mg)	≤ 600 µl

   a. For every 200 µl of sample, add 10 µl **Proteinase K**. 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 every 200 µl of the supernatant into a nuclease-free tube (not provided).
   c. Proceed to Total RNA 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 Total RNA Purification, page 10.
2 For a 1X solution of **DNA/RNA Shield™**, see Buffer Preparation, page 5.
3 For examples of sample type input and average yield, see chart on page 4.
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 (≤ 20 mg)	Bacteria (≤ $2x10^8$) Yeast (≤ $2x10^7$) Swab, Stool/Soil (≤ 10 mg)	800 µl

2. After homogenization, remove particulate debris by centrifugation at max speed. Transfer every 200 µl of the cleared supernatant into a nuclease-free tube (not provided).
3. Proceed to Total RNA Purification, page 10.

FFPE Tissue

1. Remove (trim) excess paraffin wax from ≤ 5 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 every 200 µl supernatant to a nuclease-free tube (not provided).
6. Proceed to Total RNA 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)
≤ 0.5 ml blood (≤ 10^6 cells)	200 µl

2. For every 200 µl of sample, add 10 µl **Proteinase K**. 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 every 200 µl of the cleared supernatant to a nuclease-free tube (not provided).
5. Proceed to Total RNA Purification, page 10.

Whole Blood[1] (mammalian)

1. Add 200 µl **DNA/RNA Shield™** (2X concentrate) directly to each 200 µl of fresh or frozen blood sample and mix thoroughly.
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 800 µl of the sample mixture into a new plate/tube and proceed to Total RNA 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)
100 µl (≤ 5 x 10^6 cells)	100 µl

2. For every 200 µl of reagent/sample mixture, add 10 µl **Proteinase K** and 20 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 200 µl of the cleared supernatant to a nuclease-free tube (not provided).
5. Proceed to Total Nucleic Acid Purification, page 10.

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1 Compatible with commonly used anticoagulants (e.g., EDTA, citrate, heparin)
2 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.
3 Urine Conditioning Buffer (D3061-1-8, D30601-1-140) is sold separately.
4 For a 1X solution of **DNA/RNA Shield™**, see Buffer Preparation, page 5.

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 300 µl of **RNA Lysis Buffer** to the supernatant (1:1), mix well.
6. Add 600 µl of ethanol (95-100%) to the mixture (1:1) and mix well. Proceed to Total RNA Purification, page 10, step 3.

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

1. Add 200 µl (1 volume) **RNA Lysis Buffer** to 200 µl sample and mix well[1].
2. Add 400 µl ethanol (95-100%) to the sample and mix well[1].
3. Add 30 µl **MagBinding Beads** and mix well[1] for 20 minutes.

   Important: **MagBinding Beads** settle quickly, ensure that beads are kept in suspension while dispensing.
4. Transfer the plate/tube to the magnetic stand[2] until beads have pelleted, then aspirate[3] and discard the cleared supernatant.
5. Add 500 µl **MagBead DNA/RNA Wash 1** and mix well[1]. Pellet the beads[2,3] and discard the supernatant.
6. Add 500 µl **MagBead DNA/RNA Wash 2** and mix well[1]. Pellet the beads[2,3] and discard the supernatant.
7. Add 500 µl ethanol (95-100%) and mix well[1]. Pellet the beads[2,3] and discard the supernatant.
8. Repeat step 7.
9. **DNase I** treatment (optional)
   (D1) Add 50 µl **DNase I Reaction Mix** and mix gently for 10 minutes.
   (D2) Add 500 µl **RNA Prep Buffer** and mix well[1] for 10 minutes. Pellet the beads[2,3] and discard the supernatant.
   (D3) Repeat steps 7-8.
10. Dry the beads for 10 minutes or until dry[4].
11. To elute RNA from the beads, add ≥ 50 µl **DNase/RNase-Free Water** and mix well[1] for 5 minutes.
12. Transfer the plate/tube to the magnetic stand[2] until beads have pelleted, then aspirate[3] and dispense the eluted RNA to a new plate/tube.

    The eluted RNA can be used immediately or stored frozen.

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1 For all buffer additions and incubation steps, **mix well** by pipetting the beads up and down several times and/or by shaking (vortexing) at ~1,300 rpm. Optimization may be required.
2 Use a strong-field magnetic stand or separator (e.g., ZR-96 MagStand, P1005; sold separately) until beads have pelleted.
3 Some beads will adhere to the sides of the well. When removing the supernatant, aspirate slowly to allow these beads to be pulled to the magnet as the liquid level is lowered.
4 Beads will change in appearance from glossy black when still wet to a dull brown when fully dry. Alternatively, a heat block can be used (25-55ºC).

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, step 2.

  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. Then add an equal volume of ethanol (95-100%) (e.g., 150 µl ethanol to 150 µl mixture) and mix well. Proceed to Total RNA Purification, page 10, step 3.

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 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

Automation Scripts

The ***Quick*-RNA™ MagBead** (R2132/R2133) is compatible with automated platforms. For automation scripts and related technical support, email automation@zymoresearch.com. In the subject line, please include "Automation Scripts", instrument used and the product catalog number.

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.

Ordering Information

Product Description	Catalog No.	Size
Quick-RNA™ MagBead	R2132 R2133	96 preps. 4 x 96 preps.

Individual Kit Components	Catalog No.	Amount
RNA Lysis Buffer	R1060-1-50 R1060-1-100 R1060-1-200	50 ml 100 ml 200 ml
RNA Prep Buffer	R1060-2-50 R1060-2-100	50 ml 100 ml
MagBinding Beads	D4100-2-6 D4100-2-24	6 ml 24 ml
DNase/RNase-Free Water	W1001-6 W1001-30	6 ml 30 ml
DNase I (lyophilized) (Supplied with DNA Digestion Buffer, 4 ml)	E1010 E1011	1 set (250 U) 1 set (1500 U)
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 D3001-2-20	5 mg 20 mg
MagBead DNA/RNA Wash 1 (concentrate)	R2130-1-30 R2130-1-120	30 ml 120 ml
MagBead DNA/RNA Wash 2 (concentrate)	R2130-2-20 R2130-2-80	20 ml 80 ml
Collection Plate	C2002	2 plates
Elution Plate	C2003	2 plates
96-Well Plate Cover Foil	C2007-4	4
ZR-96 MagStand	P1005	1

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.)

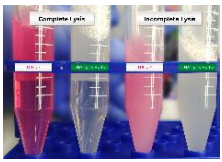
✓ For clean-up (purification) and concentration of any RNA sample. (e.g., from the aqueous phase of TRIzol® extractions) or from any enzymatic reaction (e.g., DNase I treated RNA):

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)	Incomplete lysis and/or cellular debris: - Increase the volume of DNA/RNA Shield and/or RNA Lysis Buffer for complete lysis and homogenization. Be sure to centrifuge any cellular debris and then process the cleared lysate. Washing of beads: - Shaking/Mixing: Mix well by pipetting up and down several times and/or by shaking (vortexing) at high speed. Make sure that the beads are resuspended throughout the bind, wash and elution steps.
Low yield	Sample input: - Too much input or incomplete lysis/homogenization can cause overloading 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. Increase binding time: - At all binding steps, increase binding time for an additional ≥10 minutes (e.g., 30 minutes). Depending on the amount of biomass, more time may be required to allow RNA to be sufficiently bound to beads.
DNA contamination	To remove DNA: - Perform DNase I treatment during purification (or 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

100% satisfaction guarantee on all Zymo Research products, or your money back.

Zymo Research is committed to simplifying your research with quality products and services. If you are dissatisfied with this product for any reason, please call 1(888) 882-9682.

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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