ZYMO RESEARCH

RNA Purification Made Simple™

Quick-DNA/RNA™ MagBead

DNA & RNA from any sample

Highlights

- High-throughput, magnetic-bead purification of DNA and 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 is included for unique preservation and lysis technology.
- DNA & RNA is eluted in one elution or into two separate fractions, ready for Next-Gen Sequencing, RT/qPCR, etc. *DNase I is included.*

Catalog Numbers:
R2130, R2131

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

Table of Contents

Product Contents

Quick-DNA/RNA™ MagBead	R2130 (96 prep)	R2131 (4 x 96 prep)
DNA/RNA Shield™ (2X concentrate)	25 ml	125 ml
Proteinase K[1] (lyophilized) & Storage Buffer	20 mg	20 mg (x4)
DNA/RNA Lysis Buffer	50 ml (x2)	200 ml (x2)
MagBinding Beads	6 ml	24 ml
MagBead DNA/RNA Wash 1[2] (concentrate)	30 ml (x3)	120 ml (x3)
MagBead DNA/RNA Wash 2[3] (concentrate)	20 ml (x3)	80 ml (x3)
DNase I[1] (lyophilized)	250 U (x3)	1500 U (x2)
DNA Digestion Buffer	4 ml	4 ml
DNA/RNA Prep Buffer	50 ml (x2)	200 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 4.
2 Add 20 ml (R2130) or 80 ml (R2131) of isopropanol to the **MagBead DNA/RNA Wash 1** (concentrate).
3 Add 30 ml (R2130) or 120 ml (R2131) of isopropanol to the **MagBead DNA/RNA Wash 2** (concentrate).

Specifications

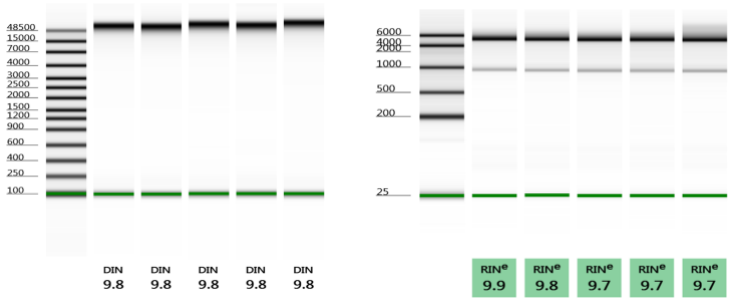
- **Sample Sources –** Any cells (animal, bacterial, etc.), solid tissue (mammalian, FFPE), biological fluids (whole blood, urine), environmental (plant/seed), swabs (stool, soil, microbial samples), samples stored in **DNA/RNA Shield™** or other preservation reagents.
- **Sample Preservation and Inactivation – 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 –** Genomic DNA (≥ 40 kb), mitochondrial and viral DNA (if present) and total RNA including small/microRNAs (≥ 17 nt).
- **Purity –** A_{260}/A_{280} & A_{260}/A_{230} > 1.8. DNA & RNA is ready for Next-Gen Sequencing, RT/qPCR, etc.
- **Binding Capacity –** 15 µg DNA/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-DNA/RNA™ MagBead** kit provides a high-throughput, magnetic bead-based purification of both high-quality DNA and total RNA (including small/microRNAs) from the same starting sample. The provided **DNA/RNA Shield™** inactivates infectious agents and is ideal for sample storage at ambient temperatures.

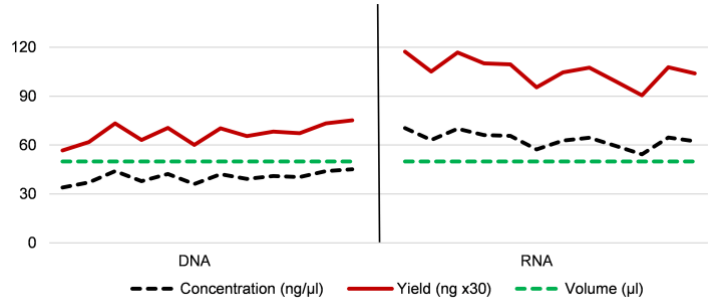
The extraction method has the option to recover total nucleic acids in one elution or DNA & RNA in separate elutions without the use of phenol. DNA/RNA is eluted into ≥ 50 µl of **DNase/RNase-Free Water** and is ready for any downstream application including Next-Gen Sequencing, RT/PCR, hybridization, *etc.*

High-Quality DNA & RNA



DNA (left) and RNA (right) quality assessed using Agilent 2200 TapeStation. DNA and RNA were purified from HeLa cells using the ***Quick*-DNA/RNA™ MagBead**.

Reproducible Sample Processing



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

Protocol

The protocol consists of: (I) Buffer Preparation, (II) Sample Preparation, (III) Total Nucleic Acid Purification and/or (IV) DNA and RNA Purification

(I) Buffer Preparation

- ✓ Add 20 ml (R2130) or 80 ml (R2131) isopropanol to the **MagBead DNA/RNA Wash 1** concentrate.
- ✓ Add 30 ml (R2130) or 120 ml (R2131) 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™[1] (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

Pellet cells by centrifugation (≤ 500 x g for 1 minute), remove the supernatant and resuspend the cell pellet in **DNA/RNA Shield™** (1X)[1,2]. (see table below). Transfer 200 µl of the sample into a nuclease-free tube and proceed to Total Nucleic Acid Purification, page 8 or DNA & RNA Purification, page 9.

Mammalian	Gram(-) bacteria	Add DNA/RNA Shield™
≤ 10^6	≤ 10^8	≥ 200 µl

Tough-to-Lyse Samples (bacteria, yeast, swab, soil[3], stool[3], solid tissue (animal, insect, plant[3], seed[3]))

1. Tough-to-lyse samples (e.g., gram(+) bacteria, tissue, etc.), can be homogenized directly in ≥ 800 µl **DNA/RNA Shield** (1X)[1,2,4] with a mortar/pestle, dounce, syringe, tissue grinder, or bead beating (recommended) with a homogenizer: high-speed (e.g., MP Bio FastPrep-24, Bertin Precellys) or low-speed (e.g., Disruptor Genie).

Input	Gram(-) bacteria (optional; easy-to-lyse)	Gram(+) bacteria	Tissue
per prep	bacteria (≤ 10^8)	bacteria (≤ 10^8) yeast (≤ 10^7) swab, stool/soil (≤ 50 mg)	animal (high yield) (≤ 10 mg) animal (low yield) (≤ 5 mg) plant/seed, insect (≤ 5 mg)
lysis beads catalog #	0.5 mm and 0.1 mm; **S6002-96-3, S6012**	0.5 mm and 0.1 mm; **S6002-96-3, S6012**	2.0 mm; **S6002-96-2, S6003**
high-speed	30 sec	5-10 min	30-60 sec
low-speed	5-10 min	20-40 min	3-5 min

Continue to page 6 for tough-to-lyse samples.

1 Samples homogenized in **DNA/RNA Shield™** can be stored frozen for later processing.
2 For a 1X solution of **DNA/RNA Shield™,** see Buffer Preparation, page 4.
3 For PCR inhibitor removal, use OneStep PCR Inhibitor Removal Kit (D6030).
4 Solid tissues should be completely submerged in **DNA/RNA Shield™**, add as needed.

2. Add 10 µl **Proteinase** K. Mix and incubate at room temperature (20-30°C). Recommended incubation time: ≥ 30 minutes (homogenized) or 2-5 hours (non-homogenized). Optimization may be required.
3. To remove particulate debris from homogenized tissue, centrifuge and transfer 200 µl of the supernatant into a new nuclease-free tube.
4. Proceed to Total Nucleic Acid Purification, page 8 or DNA & RNA Purification, page 9.

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**[1] 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**[1], and 10 µl **Proteinase K**. Mix well.
4. Incubate at 55ºC for 1 hour. Then incubate at 94ºC for 20 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. Proceed to Total Nucleic Acid Purification, page 8 or DNA & RNA Purification, page 9.

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	≥ 200 µl

2. For every 200 µl of sample, add 10 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 200 µl of the cleared supernatant to a nuclease-free tube (not provided).
5. Proceed to Total Nucleic Acid Purification, page 8 or DNA & RNA Purification, page 9.

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1 Deparaffinization Solution (D3067-1-20) and 2X Digestion Buffer (D3050-1-20) are sold separately.
2 For a 1X solution of **DNA/RNA Shield™,** see Buffer Preparation, page 4.

Whole Blood[1] (mammalian)

1. Add 200 µl **DNA/RNA Shield™** (2X concentrate) directly to each 200 µl of fresh/frozen blood sample and mix thoroughly.
2. For every 400 µl of reagent/blood mixture, add 8 µl **Proteinase K** and mix thoroughly. Incubate at room temperature (20-30°C) for 30 minutes.
3. Add an equal volume of isopropanol (1:1) and mix well.
4. Transfer 800 µl of the sample mixture into a new plate/tube and proceed to Total Nucleic Acid Purification, page 8, step 3. DNA & RNA Purification is not compatible.

Urine[2]

1. Generate pellet from up to 40 ml urine by adding 70 µl **Urine Conditioning Buffer** for every 1 ml of urine and mix by vortex. Centrifuge at 3,000 x g for 15 minutes. Discard the supernatant and leave up to 50 µl pellet.
2. Add 150 µl **DNA/RNA Shield™** (1X)[3] and resuspend the pellet by pipetting.
3. Add 10 µl **Proteinase K**. Mix and incubate at room temperature (20-30ºC) for 30 minutes.
4. Proceed to Total Nucleic Acid Purification, page 8 or DNA and RNA Purification, page 9.

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 For a 1X solution of **DNA/RNA Shield™,** see Buffer Preparation, page 4.

(III) Total Nucleic Acid Purification

1. Add 200 µl (1 volume) **DNA/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 **DNA/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 DNA/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 DNA/RNA to a new plate/tube.

    The eluted DNA/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).

(IV) DNA and RNA Purification (in two separate fractions)

1. Add 500 µl (2.5 volumes) **DNA/RNA Lysis Buffer** to the 200 µl sample and mix well[1].
2. Add 30 µl **MagBinding Beads** and mix well for 20 minutes.

   Important: **MagBinding Beads** settle quickly, ensure that beads are kept in suspension while dispensing.
3. Transfer the plate/tube to the magnetic stand[2] until beads (DNA) have pelleted, then transfer[3] the cleared supernatant (RNA) into a new plate/tube.

DNA Purification (beads)	**RNA Purification** (supernatant)
4. Add 500 µl **MagBead DNA/RNA Wash 1** and mix well[1]. Pellet the beads[2,3] and discard the supernatant.	4. Add 700 µl (1 volume) ethanol (95-100%) to the supernatant and mix well[1].
5. Add 500 µl **MagBead DNA/RNA Wash 2** and mix well[1]. Pellet the beads[2,3] and discard the supernatant.	5. Add 30 µl/well **MagBinding Beads** and mix well[1] for 10 minutes.
6. Add 500 µl ethanol (95-100%) and mix well[1]. Pellet the beads[2,3] and discard the supernatant.	6. Transfer the plate/tube to the magnetic stand[2] until beads have pelleted, then aspirate[3] and discard the cleared supernatant.
7. Repeat step 6.	7. Add 500 µl **MagBead DNA/RNA Wash 1** and mix well[1]. Pellet the beads[2,3] and discard the supernatant.
8. Dry the beads for 10 minutes or until dry[4].	8. Add 500 µl **MagBead DNA/RNA Wash 2** and mix well[1]. Pellet the beads[2,3] and discard the supernatant.

(continue DNA and RNA Purification, page 10)

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).

DNA Purification (beads)	RNA Purification (supernatant)
9. Add 50 µl **DNase/RNase-Free Water** and mix well[1] for 5 minutes.	9. Add 500 µl ethanol (95-100%) and mix well[1]. Pellet the beads[2,3] and discard the supernatant.
10. Transfer the plate/tube to the magnetic stand[2] until beads have pelleted, then aspirate[3] and dispense the eluted DNA to a new plate/tube.	10. Repeat step 9.
	11. **DNase I** treatment (optional) (D1) Add 50 µl **DNase I Reaction Mix** and mix gently for 10 minutes. (D2) Add 500 µl **DNA/RNA Prep Buffer** and mix well[1] for 10 minutes. Pellet the beads[2,3] and discard the supernatant. (D3) Repeat steps 9-10.
	12. Dry the beads for 10 minutes or until dry[4]
	13. Add 50 µl **DNase/RNase-Free Water** and mix well[1] for 5 minutes.
	14. 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 DNA and RNA can be used immediately or stored frozen.

For footnotes, see page 9.

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 ≥ month or long term at frozen temperature.

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

RNAProtect®, All Protect®: Add 3 volumes of **DNA/RNA Lysis Buffer** to 1 volume of liquid sample (3:1) and mix well and/or homogenize (e.g., see Tough-to-Lyse samples, page 5). Proceed to page 9, step 2.

RNAlater™: Add 1 volume of nuclease-free water (or PBS) to 1 volume liquid sample (1:1) and mix. Then add 4 volumes **DNA/RNA Lysis Buffer** (4:1) to 1 volume sample/water (or PBS) mixture. Mix again and proceed to page 9, step 2.

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

PAXgene®: Refer to manufacturer's instructions to remove the reagent and then proceed to Blood Cells, page 6.

Swab samples in UTM®/VTM®, saline or PBS: Remove swab and add 3 volumes of **DNA/RNA Lysis Buffer** to 1 volume sample (3:1). Mix well and proceed to page 9, 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 a volume of liquid sample and mix well. Then proceed to Samples in DNA/RNA Shield™, page 5.

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

Add 150 µl **DNA/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 again. Proceed to page 9, RNA Purification (supernatant), step 5.

(Appendices continued)

Automation Scripts

The ***Quick*-DNA/RNA™ MagBead** (R2130/R2131) 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.

Input Capacity and Average gDNA & total RNA Yield

Input	Average DNA Yield	Average RNA Yield	Kit Capacity
Cells	0.4 µg (per 10^5 cells)	1 µg (per 10^5 cells)	Up to 10^6
HeLa	6 µg	15 µg	
High Yield Tissue[1] (mouse)	≥ 3 µg (per 10 mg)	≥ 3 µg (per 10 mg)	Up to 2 mg
Spleen	5-7 µg	3-5 µg	
Liver	1.5-3 µg	4-6 µg	
Low Yield Tissue[1] (mouse)	≥ 3 µg (per 10 mg)	≤ 3 µg (per 1 mg)	Up to 5 mg
Brain, Heart	0.5-1.5 µg	0.5-1.5 µg	
Muscle	0.5-1.5 µg	0.5-2 µg	
Lung	1.5-3 µg	1-2 µg	
Intestine	1.5-3 µg	1-3 µg	
Kidney	1.5-3 µg	2-3 µg	
Whole Blood[2]	(per 100 µl)	(per 100 µl)	Up to 200 µl
Porcine	0.5-1 µg	1-2 µg	
Human	0.2-0.5 µg	0.2-1 µg	

1 Yield from tissuecan vary due to other factors such as organism type, physiological state, and growth conditions.
2 Yield from blood can vary based upon the donor, age, and/or health conditions.

Ordering Information

Product Description	Catalog No.	Size
Quick-DNA/RNA™ MagBead	R2130 R2131	96 preps. 4 x 96 preps.

Individual Kit Components	Catalog No.	Amount
DNA/RNA Lysis Buffer	D7001-1-50 D7001-1-200	50 ml 200 ml
DNA/RNA Prep Buffer	D7010-2-50 D7010-2-200	50 ml 200 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 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 D3001-2-20	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	Up to 10^6 cells, 5 mg tissue
Miniprep Plus #D7003	Up to 5 x 10^6 cells, 50 mg tissue

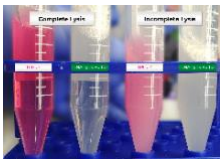
✓ For clean-up (purification) and concentration of any RNA sample. (e.g., from the aqueous phase of TRIzol® extractions) or from any enzymatic reaction (e.g., DNase I treated RNA):

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 DNA/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 DNA/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 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 DNA/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 DNA/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 DNA/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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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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