

Quick-DNA/RNA™ Miniprep Plus Kit

DNA & RNA from any sample

Highlights

- Spin-column 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 two separate fractions and is ready for Next-Gen Sequencing, RT/qPCR, etc. *DNase I is included.*

Catalog Numbers:
D7003T, D7003



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



Table of Contents

Product Contents	01
Specifications.....	02
Product Description.....	03
Protocol	04
(I) Buffer Preparation	04
(II) Sample Preparation.....	05
DNA/RNA Shield Samples, Cells, Tough-to-Lyse	05
FFPE Tissue, Blood Cells	06
Whole Blood, Urine	07
(III) DNA and RNA Purification.....	08
Appendices	09
DNA/RNA Shield Stabilization and Storage	09
RNA Protect, RNAlater, PAXgene, UTM/VTM, etc.	09
Liquids/Reaction Clean-up	10
DNase I Treatment	10
Protein Purification	10
Input Capacity and Average Total DNA/RNA Yield	11
Ordering Information	12
Complete Your Workflow.....	13
Troubleshooting Guide.....	14
Notes.....	15
Guarantee	17

Product Contents

Quick-DNA/RNA™ Miniprep Plus Kit	D7003T (10 prep)	D7003 (50 prep)
DNA/RNA Lysis Buffer	10 ml	50 ml
DNA/RNA Prep Buffer	5 ml (x2)	50 ml
DNA/RNA Wash Buffer ¹	16 ml (x2) (ready-to-use)	24 ml (x2) (concentrate)
DNase/RNase-Free Water	1 ml (x2)	10 ml
DNase I ² (lyophilized)	50 U	250 U
DNA Digestion Buffer	0.8 ml	4 ml
DNA/RNA Shield™ (2X concentrate)	5 ml	25 ml
PK Digestion Buffer	1 ml	5 ml
Proteinase K ³ (lyophilized) & Storage Buffer	5 mg	20 mg
Spin-Away™ Filters	10	50
Zymo-Spin™ IICG Columns	10	50
Collection Tubes	30	150
Instruction Manual	1 pc	1 pc

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 (D7003). **RNA Wash Buffer** (D7003T) 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**, see Buffer Preparation, page 4. Store frozen aliquots.

Specifications

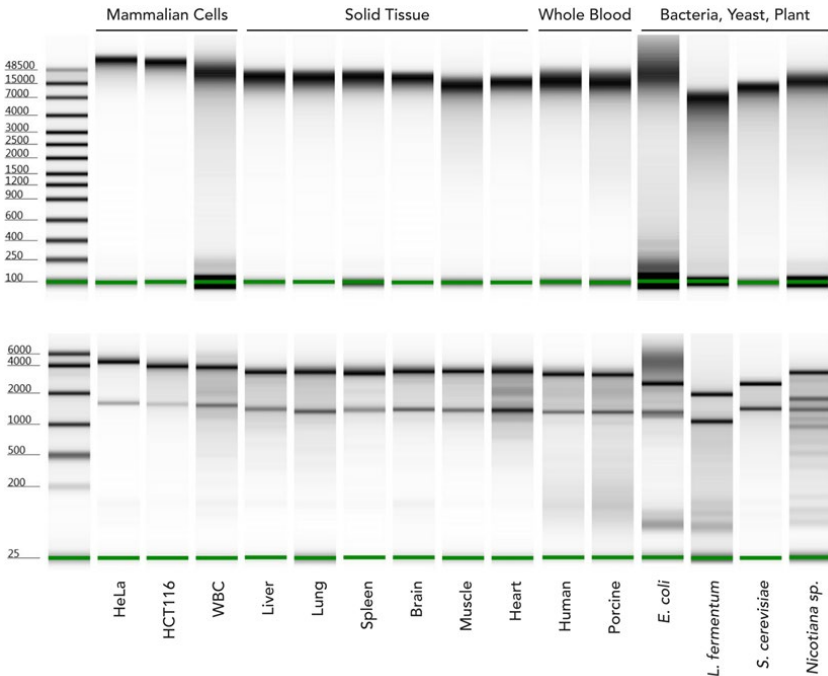
- **Sample Sources** – Any cells (animal, bacterial, blood cells, etc.), all tissues (tough-to-lyse, FFPE, etc.), blood, biological fluids, enzymatic reactions (e.g., DNase I treated) and samples in **DNA/RNA Shield™**.
- **Sample Preservation and Inactivation** – **DNA/RNA Shield™** lyses cells, inactivates nucleases and infectious agents (e.g., virus, pathogens) and is ideal for safe sample storage and transport at ambient temperatures (page 9).
- **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** – **Spin-Away Filter (yellow)** and **Zymo-Spin™ IIICG Column (green)** yield up to 100 µg DNA and RNA, respectively.
- **Compatibility** – For samples stored in preservation reagents: **DNA/RNA Shield™**, RNAprotect®, Allprotect®, Universal transport medium/viral transport medium (UTM®/VTM®), PAXgene® and RNeasy®.
- **Elution Volume** – ≥ 50 µl **DNase/RNase-Free Water**.
- **Equipment Needed** (user provided) – Microcentrifuge, vortex, heat block, water bath or incubator.

Product Description

The **Quick-DNA/RNA™ Miniprep Plus Kit** combines **Quick-DNA/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 DNA and total RNA (including small RNAs 17-200 nt) and is ready for Next-Gen Sequencing, RT/qPCR, hybridization, etc.

High-Quality DNA & RNA From Any Sample Type



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

Protocol

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

(I) Buffer Preparation

- ✓ Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA/RNA Wash Buffer** concentrate (D7003). **DNA/RNA Wash Buffer** (D7003T) is supplied ready-to-use and does not require the addition of ethanol.
- ✓ Reconstitute lyophilized **DNase I** with **DNase/RNase-Free Water** and mix by gentle inversion. Use immediately or store frozen aliquots:
#E1009-A (250 U), add 275 µl **water**
#E1009-A-S (50 U), add 55 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:
#D3001-2-20 (20 mg), add 1.04 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™).

Cells¹

- For samples in **DNA/RNA Shield™**, add an equal volume of **DNA/RNA Lysis Buffer** (1:1), mix well and proceed to purification, page 8.
- For cells, pellet by centrifugation ($\leq 500 \times g$ for 1 minute), remove the supernatant and resuspend the cell pellet in **DNA/RNA Lysis Buffer** (see table below). Proceed to purification, page 8.

Mammalian	Add DNA/RNA Lysis Buffer
$\leq 5 \times 10^6$	$\geq 300 \mu\text{l}$
$5 \times 10^6 - 10^7$	$\geq 600 \mu\text{l}$

Tough-to-Lyse Samples (bacteria, yeast, swab, soil², stool², solid tissue (animal, insect, plant², seed²))

- Tough-to-lyse samples (e.g., gram(+) bacteria, tissue, etc.), can be homogenized directly in $\geq 800 \mu\text{l}$ **DNA/RNA Shield** (1X)^{3,4} 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 ($\leq 10^9$)	bacteria ($\leq 10^9$) yeast ($\leq 10^8$) swab, stool/soil ($\leq 50 \text{ mg}$)	animal (high yield) ($\leq 25 \text{ mg}$) animal (low yield) ($\leq 50 \text{ mg}$) plant/seed, insect ($\leq 200 \text{ mg}$)
lysis beads catalog #	0.5 mm and 0.1 mm; S6012	0.5 mm and 0.1 mm; S6012	2.0 mm; 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 For cells in suspension or in other liquids/media, add ≥ 3 volumes **DNA/RNA Lysis Buffer** to 1 volume liquid sample (3:1) and mix well. Proceed to purification, page 7.

² For PCR inhibitor removal, use OneStep PCR Inhibitor Removal Kit (D6030).

³ For a 1X solution of **DNA/RNA Shield™**, see Buffer Preparation, page 4.

⁴ Solid tissues should be completely submerged in **DNA/RNA Shield™**, add as needed.

- For every 300 µl sample, add 15 µl **Proteinase K** and 30 µl **PK Digestion Buffer**. 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.
- To remove particulate debris from homogenized tissue, centrifuge and transfer the cleared supernatant into a nuclease-free tube (not provided).
- Add **DNA/RNA Lysis Buffer** to the supernatant (1:1) and mix well. Proceed to purification, page 8.

FFPE Tissue

- Remove (trim) excess paraffin wax from ≤ 25 mg FFPE tissue and transfer into a nuclease-free tube (not provided).
- Add 400 µl **Deparaffinization Solution**¹ to the sample. Incubate at 55°C for 1 minute. Vortex briefly. Remove the **Deparaffinization Solution**.
- Add 95 µl **DNase/RNase-Free Water**, 95 µl **2X Digestion Buffer**¹, and 10 µl **Proteinase K**. Mix well.
- Incubate at 55°C for 1 hour. Then incubate at 94°C for 20 minutes to de-crosslink the sample.
- Centrifuge to remove insoluble debris and transfer 200 µl supernatant to a nuclease-free tube (not provided).
- Add **DNA/RNA Lysis Buffer** to the supernatant (1:1) and mix well. Proceed to purification, page 8.

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

- 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	≥ 300 µl

- For every 300 µl of sample, add 15 µl **Proteinase K**.
- Mix and incubate at room temperature (20-30°C) for: ≥ 30 minutes. Optimization may be required.
- 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).
- Add **DNA/RNA Lysis Buffer** to the supernatant (1:1) and mix well. Proceed to purification, page 8.

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 Blood¹ (mammalian)

1. Add 200 µl **DNA/RNA Shield™** (2X concentrate) directly to each 200 µl of fresh/frozen blood sample and mix thoroughly².
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 the mixture into a **Zymo-Spin™ IIICG Column^{3,4} (green)** in a **Collection Tube** and centrifuge⁵. Discard the flow-through and proceed to purification, page 8, step 3.

Urine⁶

1. Generate pellet from up to 40 ml urine by adding 70 µl **Urine Conditioning Buffer⁷** 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)⁸ and resuspend the pellet by pipetting.
3. Add 10 µl **Proteinase K**. Mix and incubate at room temperature (20-30°) for 30 minutes.
4. Add **DNA/RNA Lysis Buffer** to the supernatant (1:1) and mix well. Proceed to purification, page 8.

1 Up to 3 ml blood per prep can be processed (with reloading the column).

2 To retain protein in the whole blood sample, omit step 2 and continue to step 3.

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

4 At this point, **DNase I** treatment (in-column) can be performed (see page 10).

5 Optional: At this point, proteins can be purified from the flow-through (page 10).

6 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.

7 Urine Conditioning Buffer (D3061-1-8, D30601-1-140) is sold separately.

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

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

- ✓ 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 **DNA/RNA Lysis Buffer** into a **Spin-Away™ Filter¹ (yellow)** in a **Collection Tube** and centrifuge. Save the flow-through for RNA purification and the filter for DNA purification!

For **whole-blood samples only**: Discard the flow-through from step 1 (above). Then transfer the **Spin-Away™ Filter** into a nuclease-free tube (not provided). Add 200 µl **DNA/RNA Lysis Buffer** directly to the matrix of the filter, let stand for 5 minutes and then centrifuge. Save the flow-through and the filter column! Then proceed to purification below.

DNA Purification

(DNA is in the filter)

- 2a. Transfer the **Spin-Away Filter¹ (yellow)** into a new **Collection Tube**.

RNA Purification

(RNA is in the flow-through)

- 2b. Add 1 volume ethanol (95-100%) to the flow-through (1:1) and mix well. Then transfer the sample into a **Zymo-Spin™ IICG Column¹ (green)** in a **Collection Tube** and centrifuge². Discard the flow-through.

At this point, **DNase I** treatment (in-column) can be performed (see page 10).

3. Add 400 µl **DNA/RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
4. Add 700 µl **DNA/RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.
5. Add 400 µl **DNA/RNA Wash Buffer** and centrifuge the column for 2 minutes to ensure complete removal of the wash buffer. Then carefully, transfer the column into a nuclease-free tube (not provided).
6. Add 100 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

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

The eluted DNA/RNA³ can be used immediately or stored frozen.

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

2 Optional: At this point, proteins can be purified from the flow-through (page 10).

3 For complete removal of PCR (RT) inhibitors from plant, soil and fecal samples, use the OneStep™ PCR Inhibitor Removal Kit (D6030).

Appendices

Samples stabilized and stored in DNA/RNA Shield™

Recommended: **DNA/RNA Shield™** effectively lyses cells, inactivates nucleases and infectious agents and is ideal for sample storage/transport at ambient temperatures prior to nucleic acid purification.

Liquid samples: Mix an equal volume **DNA/RNA Shield™** (2X concentrate) and sample (1:1).

Solid samples: Submerge sample (not to exceed 10% (v/v or w/v) in **DNA/RNA Shield™** (1X).

Mix well/homogenize sample prior to storage. Samples in **DNA/RNA Shield™** can be stored at ambient temperature ≥ 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 purification, page 8, step 2b.

✓ 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** to 1 volume sample/water (or PBS) mixture (4:1). Mix again and proceed to purification, page 8, step 2b.

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 Sample Preparation, 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 purification, page 8, step 2b.

Optional: To inactivate, store and preserve at room temperature prior to purification, add 1 volume of **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 5.

(Appendices continued)

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. Proceed to purification, page 8, step 2b.

DNase I Treatment (in-column)

1. Following RNA binding step (page 8, step 2b), add 400 µl **DNA/RNA Wash Buffer** to the column, centrifuge and discard the flow-through.
2. For each sample to be treated, prepare **DNase I Reaction Mix** in a nuclease-free tube (not provided) and mix by gentle inversion. Then add 80 µl directly into column matrix and incubate at room temperature (20-30°C) for 15 minutes. Proceed with the purification protocol (page 8, step 3).

DNase I Reaction Mix

DNase I (reconstituted; 1 U/ul) ¹	5 µl
DNA Digestion Buffer	75 µl

Protein Purification: Acetone Precipitation of Proteins

- ✓ After the RNA binding to the column (page 8, step 2b), the protein content in the flow-through can be purified:
1. Add 4 volumes of cold acetone (-20°C) to the flow-through (4:1) and mix.
 2. Incubate the samples for 30 minutes on ice.
 3. Centrifuge at max speed for 10 minutes. Discard the supernatant. Keep the pellet.
 4. Add 400 µl ethanol (95-100%) to the protein pellet. Centrifuge at max speed for 1 minute. Discard the supernatant.
 5. Air-dry the protein pellet for 10 minutes at room temperature.
 6. Resuspend and vortex the pellet in a buffer appropriate for downstream application (e.g., SDS-PAGE sample loading buffer).

¹ Unit definition – one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A₂₆₀ units/ml of reaction mixture at 25°C.

(Appendices continued)

Input Capacity and gDNA and total RNA Yield

Input	Average gDNA Yield	Average RNA Yield	Kit Capacity
Cells	4 µg (per 10 ⁶ cells)	10 µg (per 10 ⁶ cells)	Up to 10 ⁷
HeLa	6 µg	15 µg	
High Yield Tissue ^{1 (mouse)}	≥ 30 µg (per 10 mg)	≥ 30 µg (per 10 mg)	Up to 20 mg
Spleen	50-70 µg	30-50 µg	
Liver	15-30 µg	40-60 µg	
Low Yield Tissue ^{1 (mouse)}	≥ 30 µg (per 10 mg)	≤ 30 µg (per 10 mg)	Up to 50 mg
Brain, Heart	5-15 µg	5-15 µg	
Muscle	5-15 µg	5-20 µg	
Lung	15-30 µg	10-20 µg	
Intestine	15-30 µg	10-30 µg	
Kidney	15-30 µg	20-30 µg	
Whole Blood ²	(per 1 ml)	(per 1 ml)	Up to 3 ml
Porcine	5-10 µg	10-20 µg	
Human	2-5 µg	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.

Ordering Information

Product Description	Catalog No.	Size
Quick-DNA/RNA™ Miniprep Plus Kit	D7003T D7003	10 preps. 50 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	D7010-2-25 D7010-2-50	25 ml 50 ml
DNA/RNA Wash Buffer (concentrate)	D7010-3-12 D7010-3-24	12 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
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.)

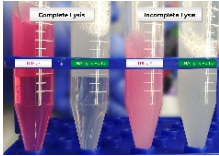
- ✓ For clean-up (purification) and concentration of any RNA sample. (e.g., from the aqueous phase of TRIzol[®] extractions) or from any enzymatic reaction (e.g., DNase I treated RNA):

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	<p>Incomplete lysis and/or high-mass input:</p> <ul style="list-style-type: none"> - If precipitation occurs (upon adding ethanol to the lysate) or if the lysate is extremely viscous, increase the volume of DNA/RNA Shield™ and/or 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)	<p>Sample handling:</p> <ul style="list-style-type: none"> - Ethanol and/or salt contamination. After centrifugation steps, carefully remove the column from the collection tube to prevent buffer carryover. Alternatively, blot emptied collection tube with a tissue or towel. - Make sure lysate and wash buffers have passed completely through the matrix of the column. This may require centrifuging at a higher speed and/or longer time. <p>Incomplete lysis and/or cellular debris:</p> <ul style="list-style-type: none"> - Increase the volume DNA/RNA Shield™ and/or DNA/RNA Lysis Buffer (proportionally) to ensure complete lysis and homogenization. Be sure to centrifuge any cellular debris and then process the cleared lysate.
Low yield	<p>Sample input:</p> <ul style="list-style-type: none"> - Too much input or incomplete lysis/homogenization can cause cellular debris to clog or overload the column and result in compromised nucleic acid recovery. Use less input material and/or increase the volume DNA/RNA Shield™ and/or DNA/RNA Lysis Buffer. <p>High-protein content (blood, plasma/serum, etc.)</p> <ul style="list-style-type: none"> - Perform Proteinase K treatment to the sample prior to purification. See appropriate sample preparation protocol.
DNA contamination	<p>To remove DNA:</p> <ul style="list-style-type: none"> - Perform in-column DNase I treatment (page 8) 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 DNA/RNA Lysis Buffer to ensure complete lysis and homogenization of the sample.
RNA degradation	<p>To prevent RNA degradation:</p> <ul style="list-style-type: none"> - 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

This image shows a single sheet of white paper with horizontal ruling lines. The lines are evenly spaced and run across the width of the page. There are no margins, text, or other markings on the paper.

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