ZYMO RESEARCH

RNA Purification Made Simple™

Direct-zol™-96 MagBead RNA

TRIzol® In. RNA Out.

Highlights

- High-throughput, magnetic bead-based purification of total RNA (including small/microRNAs) ***directly*** from TRIzol®, TRI Reagent® or similar acid-guanidinium-phenol based reagents.
- No need for chloroform, phase-separation or precipitation steps.
- RNA is ready for Next-Gen Sequencing, RT-qPCR, etc. *DNase I is included.*

Catalog Numbers:
R2100, R2101, R2102, R2103

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

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

Table of Contents

Product Contents

Direct-zol™-96 MagBead RNA	R2100 (96)	R2101 (96)	R2102 (4 x 96)	R2103 (4 x 96)
TRI Reagent®	-	50 ml (x2)	-	200 ml (x2)
MagBinding Beads	3 ml	3 ml	12 ml	12 ml
MagBead DNA/RNA Wash 1[1] (concentrate)	30 ml (x2)	30 ml (x2)	120 ml (x2)	120 ml (x2)
MagBead DNA/RNA Wash 2[2] (concentrate)	20 ml (x2)	20 ml (x2)	80 ml (x2)	80 ml (x2)
RNA Prep Buffer	100 ml (x2)	100 ml (x2)	100 ml (x4)	100 ml (x4)
DNase I[3] (lyophilized)	250 U (x3)	250 U (x3)	1500 U (x2)	1500 U (x2)
DNA Digestion Buffer	4 ml	4 ml	4 ml	4 ml
DNase/RNase-Free Water	30 ml	30 ml	100 ml	100 ml
Instruction Manual	1 pc	1 pc	1 pc	1 pc

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature.
Before use:
1 Add 20 ml (R2100, R2101) or 80 ml (R2102, R2103) of isopropanol to the **MagBead DNA/RNA Wash 1** concentrate and mix well.
2 Add 30 ml (R2100, R2101) or 120 ml (R2102, R2103) of isopropanol to the **MagBead DNA/RNA Wash 2** concentrate and mix well.
3 Reconstitute lyophilized **DNase I** according to Buffer Preparation, page 4.

Specifications

- **Sample Sources –** Any sample stored and preserved in TRIzol®, TRI Reagent® or similar[1]. (animal cells, tissue, bacteria, yeast, biological fluids, samples stored in DNA/RNA Shield™ and in-vitro processed RNA (e.g., transcription products, DNase-treated or labeled RNA)).

- **Sample Inactivation –** TRI Reagent® (provided with **R2101** & **R2103** only) inhibits RNase activity and inactivates viruses and other infectious agents.

- **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 –** 10 µg RNA per 20 µl **MagBinding Beads**.

- **Compatibility –** TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ or similar acid-guanidinium-phenol based reagents can be used in place of TRI Reagent®.

  Also, compatible with samples in TRIzol®, TRI Reagent® or similar reagent that contain chloroform, 1-bromo-3-chloropropane (BCP), or 4-bromoanisole (BAN).

- **Elution Volume –** ≥ 50 µl **DNase/RNase-Free Water**.

- **Recommended Materials** (available separately): ZR-96 MagStand (P1005), Collection Plate (C2002; capacity 1.2 ml/well), 96-Well Block (P1001; capacity 2 ml/well), Elution Plate (C2003; capacity 0.35 ml/well), 96-Well Plate Cover Foil (C2007; 2, 6, 8 pack), DNase/RNase-Free Tubes (C2001; 100 pack).

- **Equipment Needed** (user provided): Magnetic stand or separator, liquid handler or robotic sample processor.

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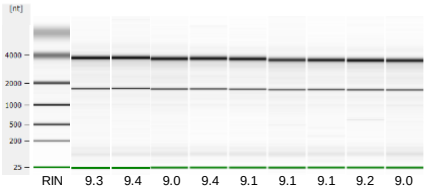
1 RNAzol®, QIAzol®, TriPure™, TriSure™ or similar acid-guanidinium-phenol reagent.

Product Description

The **Direct-zol™-96 MagBead RNA** kit provides a high-throughput, magnetic bead-based isolation of high-quality RNA *directly* from samples in TRIzol®, TRI Reagent® or similar reagent[1]. The extraction method inactivates viruses and other infectious agents[2]. Total RNA (including small/microRNAs) are effectively isolated from a variety of sample sources (cells, tissues, biological liquids, DNA/RNA Shield™ samples, *etc.*).

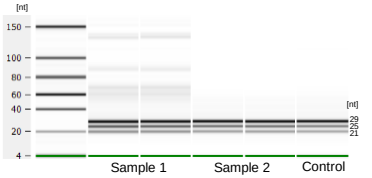
The procedure is easy: simply add ethanol and **MagBinding Beads** to a sample in TRIzol®/TRI Reagent®, wash and elute the RNA. No chloroform, phase separation or precipitation steps are necessary. High-quality total RNA is ready for Next-Gen sequencing, RT-qPCR, transcription profiling, hybridization, *etc*.

High-quality total RNA



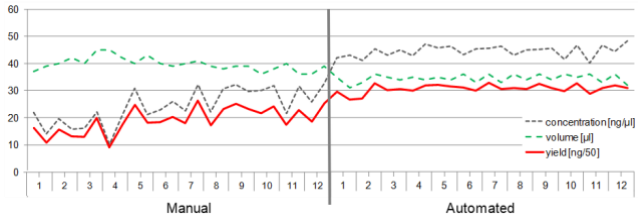
RNA quality (RIN) assessed using a Bioanalyzer. RNA was purified from epithelial cell using the the **Direct-zol™-96 MagBead RNA** kit on Freedom EVO® (Tecan).

Efficient small RNA recovery



Small RNA recovery with the **Direct-zol™-96 MagBead RNA** kit. (Small RNA Chip gel image shown; Bioanalyzer).

Reproducible sample processing



Comparison between manual and automated (Freedom EVO®, Tecan) sample processing with the **Direct-zol™-96 MagBead RNA** kit across a 96-well plate. RNA was purified from human epithelial cells (5x 10^5/well).

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1 RNAzol®, QIAzol®, TriPure™, TriSure™ or similar acid-guanidinium-phenol reagent.
2 TRI Reagent® provided with catalog #R2101 and #R2103.

Protocol

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

The following guidelines are provided for processing various sample types in TRIzol®, TRI Reagent® or similar[1] acid-guanidinium-phenol reagents prior to column purification of the RNA (see page 7 for Input Capacity and Average RNA Yield).

(I) Buffer Preparation

- ✓ Add 20 ml (R2100, R2101) or 80 ml (R2102, R2103) of isopropanol to the **MagBead DNA/RNA Wash 1** concentrate and mix well.
- ✓ Add 30 ml (R2100, R2101) or 120 ml (R2102, R2103) of isopropanol to the **MagBead DNA/RNA Wash 2** concentrate and mix well.
- ✓ 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[2]

- ✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 1 minute.

Cells

Lyse animal or gram(-) bacteria cells* directly in a culture dish** or resuspend pelleted cells in an appropriate volume (see table below) of TRI Reagent® or similar[1] and mix thoroughly. Centrifuge to remove debris (if any) and transfer the cleared supernatant into a nuclease-free tube (not provided). Proceed to RNA Purification (page 6).

Animal	Gram(-) bacteria	Add TRI Reagent®
≤ 10^5	-	≥ 100 µl
≤ 10^6	≤ 10^8	≥ 300 µl

* For cell suspensions, add 3 volumes of TRI Reagent® to 1 volume of cell suspension.
** For direct lysis in a dish, add 100 µl for each cm^2 of culture surface area.

1 TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ or similar acid-guanidinium-phenol reagent.
2 RNA yield can vary with sample types, organism, quality and treatment of the starting material (see page 7). To ensure complete lysis and homogenization of a sample, use a sufficient amount of TRIzol®, TRI Reagent® or similar reagent. For detailed processing information, refer to the TRI Reagent® product manual (or manufacturer's instructions for the reagent used).

Tough-to-lyse samples

Tough-to-lyse samples (see table below) can be homogenized in ≥ 800 µl TRIzol®, TRI Reagent® or similar[1] with a mortar/pestle, dounce, syringe, tissue grinder, or bead beating with a high-speed homogenizer.

To remove particulate debris from homogenized tissue, centrifuge and transfer the supernatant into a new nuclease-free tube. Proceed to RNA Purification (page 6).

Recommended: Use ZR BashingBead™ Lysis Tubes (materials sold separately; #S6012, #S6003, #S014) for complete lysis and homogenization.

Input	Gram(-) bacteria (optional; easy-to-lyse)	Gram(+) bacteria	Tissue	Pathogen (microbes in tissue)
per prep	bacteria (≤ 10^8)	bacteria (≤ 10^8) yeast (≤ 10^7)	animal: high yield (≤ 5 mg) animal: low yield (≤ 10 mg) plant (≤ 100 mg)	animal/insect, plant (≤ 5 mg)
lysis beads catalog #	0.5 mm and 0.1 mm; **S6012**	0.5 mm and 0.1 mm; **S6012**	2.0 mm; **S6003**	2.0 mm and 0.1 mm; **S6014**
high-speed[2,3]	30 sec	5-10 min	30-60 sec	3-5 min
low-speed[3]	5-10 min	20-40 min	3-5 min	5-10 min

Liquids

Add an appropriate volume of TRI Reagent® or similar[1] to a liquid sample and mix thoroughly (see table below). To remove particulate debris (if any), centrifuge and transfer the supernatant into an RNase-free tube. Proceed to RNA Purification (page 6).

Recommended: For biological samples (whole-blood, plasma, serum, buffy coat, PBMCs, WBCs, FACS, etc.) or samples collected in DNA/RNA Shield™[4], perform Proteinase K treatment[5] (sold separately) prior to adding TRI Reagent®.

Sample		Add TRI Reagent®
Biological liquid (blood, plasma, serum, WBCs, FACs, etc.) or **Reaction clean-up** (DNase I treated RNA, *in vitro* transcription, labeling, etc.).	100 µl	≥ 300 µl
Samples in DNA/RNA Shield™ (biological sample[4,5] or stored purified RNA).	100 µl	100 µl

1 ZR-96 BashingBead™ Lysis Rack (2.0 mm) (S6002-96-2) and ZR BashingBead™ Lysis Tubes (2.0 mm) (S6003-50) are sold separately.
2 High-speed homogenizers (e.g. Precellys, FastPrep®), process for ≤ 5 minutes. Low-speed homogenizers (e.g., Disruptor Genie), process for ≥ 10 minutes.3 High-speed homogenizers (e.g., MP Bio FastPrep-24™, Bertin Precellys, etc.). Low-speed homogenizers (e.g., Disruptor Genie, etc.).
3 DNA/RNA Shield™ reagent (R1100, R1200) or DNA/RNA Shield™ Blood Collection Tube (R1150).
4 For Proteinase K treatment, see page 7.

(III) RNA Purification

1. Add an equal volume ethanol (95-100%) to a sample lysed in TRIzol, TRI Reagent® or similar reagent and mix well[1].

2. Add 20 µl **MagBinding Beads** and mix well[1] for 10 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 have pelleted, then aspirate[3] and discard the cleared supernatant.

4. Add 500 µl **MagBead DNA/RNA Wash 1** and mix well[1]. Pellet the beads[2,3] and discard the supernatant.

5. Add 500 µl **MagBead DNA/RNA Wash 2** and mix well[1]. Pellet the beads[2,3] and discard the supernatant.

6. Add 500 µl ethanol (96-100%) and mix well[1]. Pellet the beads[2,3] and discard the supernatant.

7. Repeat step 6.

8. **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 for 10 minutes. Pellet the beads[2,3] and discard the supernatant.
   (D3) Repeat steps 6-7.

9. Dry the beads for 10 minutes or until fully dry[5].

10. To elute RNA from the beads, add 50 µl **DNase/RNase-Free Water** and mix well[1] for 5 minutes.

11. 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 (or tube). When removing the supernatant, aspirate slowly to allow these beads to be pulled to the magnet as the liquid level is lowered.
4 Before use, add ethanol to the buffer concentrate (Buffer Preparation, page 4).
5 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 (25-55°C).

Appendices

Automation Scripts

Direct-zol™-96 MagBead RNA (R2100-R2103) is compatible with any automated platform. 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.

Automated platforms scripted with Direct-zol RNA™ MagBead kit: Tecan® Fluent, Hamilton®: STAR, STARlet, Kingfisher®: Flex, Duo Prime, Presto, EpMotion® 5073 (also compatible with other EpMotion devices).
*For automation platforms with "dead volume" liquid handler dispensing, additional reagents are available separately (page 8).

Input Capacity and Average 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 10 mg)	Up to 5 mg
Spleen	3-5 µg	
Liver	4-6 µg	
Low Yield Tissue[1] (mouse)	≤ 3 µg (per 1 mg)	Up to 10 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	

Proteinase K Treatment

- ✓ Proteinase K treatment can be performed on protein-rich samples stored in **DNA/RNA Shield™** (2X concentrate; #R1200) (e.g., tissue, blood cells, plasma, serum, saliva, sputum, etc.) using **Proteinase K Set** (#D3001-2-5, D3001-2-20; sold separately).

Add 10 µl Proteinase K (reconstituted) to 1 ml DNA/RNA Shield sample (scale proportionally) and mix by inversion. Then incubate at room temperature (20-30°C) for 30 minutes (homogenized) or 2-5 hours (non-homogenized). Optimization may be required.

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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
Direct-zol™-96 MagBead RNA (TRI Reagent® not included)	R2100 R2102	2 x 96 preps. 4 x 96 preps.
Direct-zol™-96 MagBead RNA (supplied with TRI Reagent®)	R2101 R2103	2 x 96 preps. 4 x 96 preps.

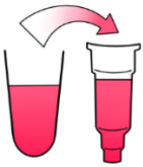
Individual Kit Components	Catalog No.	Amount
TRI Reagent®	R2050-1-50 R2050-1-200	50 ml 200 ml
MagBead DNA/RNA Wash 1 (concentrate)	R2130-1-30 R21301-120	30 ml 120 ml
MagBead DNA/RNA Wash 2 (concentrate)	R2130-2-20 R2130-2-80	20 ml 80 ml
MagBinding Beads	D4100-2-3 D4100-2-12	3 ml 12 ml
RNA Prep Buffer	R1060-2-100	100 ml
DNase/RNase-Free Water	W1001-30 W1001-100	30 ml 100 ml
DNase I (lyophilized) (250 U supplied with DNA Digestion Buffer, 4 ml)	E1010	1 set
ZR-96 MagStand	P1005	1
Collection Plate (capacity 1.2 ml/well)	C2002	2 plates
96-Well Block (capacity 2 ml/well)	P1001-2	2 plates
Elution Plate (capacity 0.35 ml/well)	C2003	2 plates
96-Well Plate Cover Foil	C2007-2	2
DNA/RNA Shield™ (2X concentrate)	R1200-25 R1200-125	25 ml 125 ml
Proteinase K Set (w/ Storage Buffer)	D3001-2-5 D3001-2-20	5 mg 20 mg

Complete Your Workflow

✓ For tough-to-lyse samples in TRIzol, use ZR BashingBead Lysis Tubes:

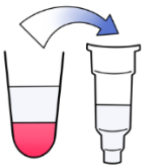
ZR BashingBead Lysis Tubes	
2.0 mm beads #S6003	For plant/animal tissue
0.1 + 0.5 mm beads #S6012	For microbes
0.1 + 2.0 mm beads #S6014	For microbes in tissue/insects

✓ The only **direct**, high-throughput and automatable RNA purification from sample lysates in TRIzol (DNase I Set included with all formats):



Direct-zol RNA kits	
Microprep #R2060-R2063	From 1 cell and up
Miniprep #R2050-R2053	Up to 50 ug RNA
Miniprep Plus #R2070-R2073	Up to 100 ug RNA
96-well #R2054-R2057	Spin-plate
MagBeads #R2100-R2105	Automatable (Tecan, Hamilton, Kingfisher, etc.)

✓ For RNA clean-up (purification) from the aqueous phase (e.g., TRIzol, TRI Reagent or similar) or from any enzymatic reaction (e.g., DNase I treated RNA):

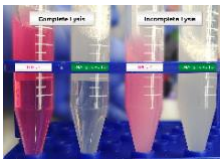


RNA Clean & Concentrator kit	
#R1013-R1014	DNase I Set included

✓ For NGS:

Zymo-Seq RiboFree Total RNA Library Prep kit	
#R3000	12 preps
#R3003	96 preps

Troubleshooting Guide

Problem	Possible Causes and Suggested Solutions
Low recovery	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 TRIzol®, TRI Reagent® or similar reagent 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 TRIzol®, TRI Reagent® or similar to ensure 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 RNA recovery. Use less input material and/or increase TRIzol®, TRI Reagent® or similar reagent. High-protein content (blood, plasma/serum, etc.) - Perform Proteinase K treatment to the sample prior to adding TRIzol®, TRI Reagent® or similar reagent (Sample preparation, page 5). 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 post-purification (R1013, page 4), then re-purify the treated sample. - For future preps, increase the volume of TRIzol®, TRI Reagent® or similar reagent to ensure complete lysis and homogenization of the sample.
RNA degradation	To prevent RNA degradation: - Immediately collect and lyse fresh sample into TRIzol®, TRI Reagent® or similar reagent to ensure RNA stability. Homogenized samples in TRIzol®, TRI Reagent® or similar can be stored frozen for later processing.

For technical assistance, please contact 1-888-882-9682 or email tech@zymoresearch.com

Notes

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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