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INSTRUCTION MANUAL

Direct-zol™-96 MagBead RNA

Catalog Nos. **R2100, R2101, R2102 & R2103**

Highlights

- High-throughput (96-well), magnetic-bead based isolation of total RNA including small/microRNAs ***directly*** from TRIzol®, TRI Reagent® and other similar reagents.
- No need for chloroform, phase separation and precipitation procedures.
- RNA is ready for Next-Gen sequencing, RT-PCR and other downstream applications. *DNase I included.*

Contents

U.S. Patent No. 9,051,563 and other pending patents.

Ver. 2.1.0

ZYMO RESEARCH CORP.

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Satisfaction of all Zymo Research products is guaranteed. If you are dissatisfied with this product, please call 1-888-882-9682.

This product is for research 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.

Storage Temperature – Store kit and components (e.g., reagents) at room temperature, unless specified.

* TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ and all other *acid-guanidinium-phenol* reagents.

Following applicable federal, state and local regulations for phenol waste disposal.

Product Contents

Direct-zol™-96 MagBead RNA (Kit Size)	**R2100** (2x 96 preps)	**R2101*** (2x 96 preps)	**R2102** (4x 96 preps)	**R2103*** (4x 96 preps)
TRI Reagent®	-	200 ml	-	2x 200 ml
MagBinding Beads	6 ml	6 ml	12 ml	12 ml
MagBead DNA/RNA Wash 1 (concentrate)[1]	2x 30 ml	2x 30 ml	120 ml	120 ml
MagBead DNA/RNA Wash 2 (concentrate)[2]	2x 20 ml	2x 20 ml	80 ml	80 ml
RNA Prep Buffer	100 ml	100 ml	2x 100 ml	2x 100 ml
DNase I[3] (250 U)	4	4	8	8
DNA Digestion Buffer	4 ml	4 ml	4 ml	4 ml
DNase/RNase-Free Water	30 ml	30 ml	2x 30 ml	2x 30 ml
Instruction Manual	1	1	1	1

TRI Reagent® is provided only with catalog numbers **R2101** & **R2103**.
[1] Add 20 mL (R2100, R2101) or 80 ml (R2102, R2103) of isopropanol to the **MagBead DNA/RNA Wash 1** concentrate.
[2] Add 30 mL (R2100, R2101) or 120 ml (R2102, R2103) of isopropanol to the **MagBead DNA/RNA Wash 2** concentrate.
[3] Prior to use, reconstitute the lyophilized **DNase I**. See instructions (page 3).

Specifications

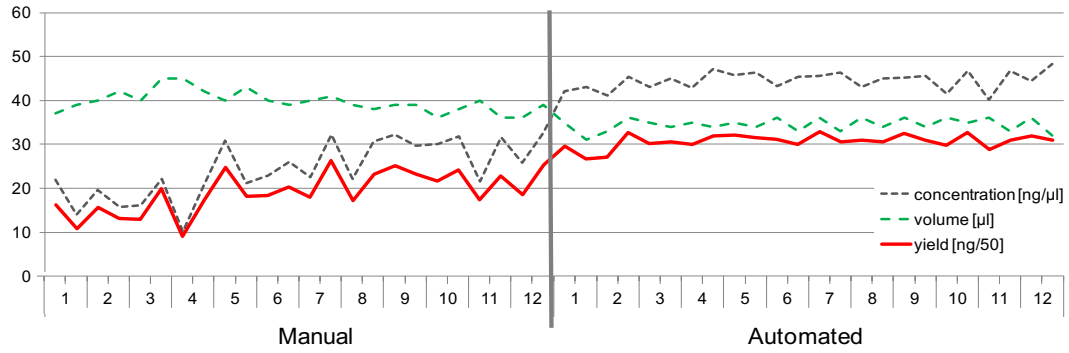
- **Sample Sources** – Any sample stored and preserved in TRIzol®, TRI Reagent®, or similar*: animal cells, tissue and biological liquids (*e.g.,* blood, plasma, serum). Also, compatible with *in vitro* processed RNA (*e.g.,* transcription products, DNase-treated or labeled RNA) and samples in DNA/RNA Shield™.
- **Purity** – High-quality RNA is ready for Next-Gen sequencing, RT-PCR, hybridization, etc. Complete removal of DNA is performed with DNase I digestion (page 4).
- **Binding Capacity** – 10 µg RNA per 20 µl magnetic beads
- **Size** – Total RNA including small/microRNAs (>17 nt)
- **Elution Volume** - ≥50 µl DNase/RNase-Free Water
- **Sample Inactivation** – TRI Reagent® (provided with R2101 & R2103 only) inhibits RNase activity and inactivates viruses and other infectious agents.
- **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)

Product Description

The **Direct-zol™-96 MagBead RNA** kit provides a high-throughput (96-well), magnetic bead-based isolation of high-quality RNA *directly* from samples in TRI Reagent® and similar[1]. The extraction method inactivates viruses and other infectious agents[2]. Total RNA including small/microRNAs (>17 nt) are effectively isolated from a variety of sample sources (cells, tissues, biological liquids, *etc.*).

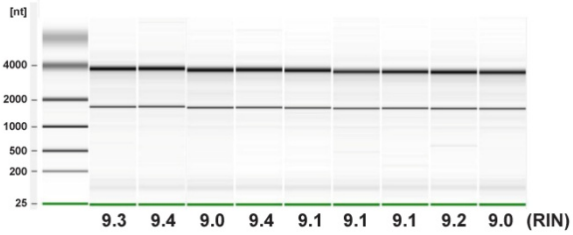
The procedure is easy: simply add ethanol and **MagBinding Beads** to a sample in TRI Reagent®, wash and elute the RNA. No phase separation, precipitation, or post-purification steps are necessary. The result is broad range purification of small and large RNAs suitable for subsequent RNA-based methods including Next-Gen sequencing, RT-PCR, transcription profiling, hybridization, *etc.*

Reproducible Sample Processing



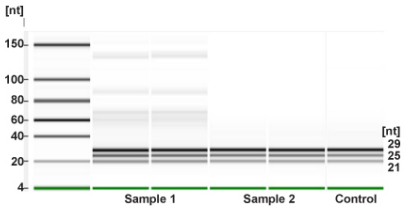
Comparison between manual and automated (Freedom EVO®, Tecan) sample processing with the **Direct-zol™-96 MagBead RNA** across a 96-well plate. RNA was purified from human epithelial cells (5x 10^5/well).

High-Quality RNA



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

Efficient Small RNA Recovery



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

For **Technical Assistance**, please contact Zymo Research Technical Support at 1-888-882-9682 or e-mail tech@zymoresearch.com.

Note:

[1] TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ and other *acid-guanidinium-phenol* reagents.

[2] For Catalog Nos. R2101 & R2103 supplied with TRI Reagent®.

Catalog Nos. R2100 & R2102 do not include TRI Reagent®.

Make sure guidelines are followed to ensure the RNA isolation procedure is performed in an RNase-free environment.

Reagent Preparation

- ✓ Add 20 mL (R2100, R2101) or 80 ml (R2102, R2103) of isopropanol to the **MagBead DNA/RNA Wash 1** concentrate.
- ✓ Add 30 mL (R2100, R2101) or 120 ml (R2102, R2103) of isopropanol to the **MagBead DNA/RNA Wash 2** concentrate.
- ✓ Prepare **DNase I Reaction Mix** (according to the example below; scale as needed):

DNase I (250 U/vial; lyophilized)	DNase/RNase-Free Water	DNA Digestion Buffer
1 vial	2.7 ml	0.3 ml
2 vials (96-well plate)	5.4 ml	0.6 ml

1. Reconstitute lyophilized **DNase I** with **DNase/RNase-Free Water** (table above), transfer into a nuclease-free tube (not provided) and mix by inversion. At this point, aliquots can be stored frozen.
2. Add **DNA Digestion Buffer** (table above) and mix by inversion, then place on ice until ready to use (page 4 RNA Purification, step 6).

Unit definition – one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A_{260} units/min/ml of reaction mixture at 25°C.

Protocol

This protocol consists of two parts: (I) Sample Preparation and (II) RNA Purification.

The following guidelines are provided for processing various sample types in TRI Reagent®, TRIzol® or similar[1] *acid-guanidinium-phenol* reagents prior to purification of the RNA. RNA yield can vary with sample types, organism, quality and treatment of the starting material. To ensure complete lysis and homogenization of a sample, use a sufficient amount of TRI Reagent® or similar.

Notes:

[1] RNAzol®, QIAzol®, TriPure™, TriSure™ and all other *acid-guanidinium-phenol* reagents.

(I) Sample Preparation

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

1. To lyse a sample, resuspend cells or homogenize tissue in an appropriate volume of TRI Reagent®, TRIzol® or similar[1] acid-guanidinium-phenol reagents (see table below). For DNA/RNA Shield™ samples and/or biological liquids (whole-blood, plasma, serum, WBCs, FACS, etc.), Proteinase K treatment[2] is recommended prior to adding TRI Reagent®.

Animal	Tissue	Liquids (blood, plasma, serum, DNA/RNA Shield™ samples, etc.)	Add TRI Reagent®
≤ 10^6	≤ 5 mg	≤ 100 µl	300 µl

2. To remove particulate debris or precipitation, centrifuge and transfer up to 200 µl of the cleared supernatant into a well/collection plate[3] or nuclease-free tube (not provided). Proceed to RNA Purification (page 4).

[2] Add 10 µl **Proteinase K** (D3001-2-5, D3001-2-20) for every 1 ml DNA/RNA Shield™/sample mixture. Mix thoroughly and incubate for 30 minutes at room temperature (20-30°C).

[3] 96-well **Collection Plate** (C2002; capacity is up to 1.2 ml/well) or **96-Well Block** (P1001; capacity is up to 2 ml/well).

(II) RNA Purification

Protocol applicable for manual processing, liquid handling and bead moving platforms (scripts available). Perform all steps at room temperature (20-30°C), unless specified.

1. Add an equal volume of ethanol (95-100%) to a sample in TRIzol® 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 or tube (not provided) to the magnetic stand[2] (sold separately) until beads have pelleted, then aspirate[3] and discard the cleared supernatant.
4. Optional: **DNase I**[5] treatment

   (D1) Add 500 µl ethanol (95-100%) and mix well. Pellet the beads, aspirate[3], discard the supernatant and repeat this wash step.
   (D2) Add 50 µl **DNase I Reaction Mix** and mix gently at room temperature for 10 minutes.
   (D3) Add 500 µl **RNA Prep Buffer** and mix well for 10 minutes. Pellet the beads, aspirate[3] and discard the supernatant. Proceed to step 5 below.
5. Add 500 µl **MagBead DNA/RNA Wash 1**[4] and mix well[1]. Pellet the beads, aspirate[3] and discard the supernatant.
6. Add 500 µl **MagBead DNA/RNA Wash 2**[4] and mix well[1]. Pellet the beads, aspirate[3] and discard the supernatant.
7. Add 500 µl ethanol (95-100%) and mix well[1]. Pellet the beads, aspirate[3], discard the supernatant and repeat this wash step.
8. Dry the beads at room temperature for 10 minutes or until fully dry[6].
9. Add 50 µl of **DNase/RNase-Free Water** and mix well for 5 minutes.

   Alternatively, for highly concentrated RNA use ≥30 µl volume.
10. Pellet the beads, aspirate[3] and dispense the eluted RNA into a new well/elution plate[7] or nuclease-free tube (not provided).

    The eluted RNA[7] can be used immediately or stored frozen.

Notes:

[1] For all buffer additions, **mix well** by pipetting up and down several times to ensure magnetic beads are all in suspension. Alternatively, vortexing at ~1,300 rpm can be used.

[2] Magnetic stand (manual processing) or strong-field 96-well magnetic stand (i.e., **ZR-96 MagStand**, P1005).

[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 isopropanol to the wash buffer concentrate (see Reagent Preparation, page 3).

[5] Before use, reconstitute the lyophilized **DNase I** (see Reagent Preparation, page 3). Store frozen aliquots.

[6] 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).

[7] **Elution Plate** (C2003; capacity is up to 0.35 ml/well). To prevent evaporation of eluted RNA, **96-Well Plate Cover Foil** (C2007) is recommended.

Automation Scripts

The **Direct-zol™-96 MagBead RNA** kit is compatible with any automated platform (e.g. Tecan Hamilton, ThermoFisher, Eppendorf). 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.

Appendix

RNA extraction from samples stored in DNA/RNA Shield™

Add TRIzol® or similar to each sample in DNA/RNA Shield™ (1:1) and mix thoroughly. To remove particulate debris, centrifuge (12,000 x g for 1 minute) and transfer the supernatant into an RNase-free tube/plate. Proceed to RNA Purification (page 4).

Ordering Information

Product Description	Kit Size	Catalog No.
Direct-zol™-96 MagBead RNA (TRI Reagent® not included)	2x 96 preps. 4x 96 preps.	R2100 R2102
Direct-zol™-96 MagBead RNA (supplied with TRI Reagent®)	2x 96 preps. 4x 96 preps.	R2101 R2103

Individual Kit Components	Amount	Catalog No.
TRI Reagent®	50 ml 200 ml	R2050-1-50 R2050-1-200
MagBead DNA/RNA Wash 1 (concentrate)	30 ml 120 ml	R2130-1-30 R2130-1-120
MagBead DNA/RNA Wash 2 (concentrate)	20 ml 80 ml	R2130-2-20 R2130-2-80
DNase/RNase-Free Water	10 ml 30 ml	W1001-10 W1001-30
DNase I (lyophilized) (250 U supplied with DNA Digestion Buffer, 4 ml)	1 set	E1010
MagBinding Beads	3 ml 6 ml 12 ml	D4100-2-3 D4100-2-6 D4100-2-12
RNA Prep Buffer	100 ml	R1060-2-100
96-Well Plate Cover Foil	2 6 8	C2007-2 C2007-6 C2007-8
Collection Plate (capacity 1.2 ml/well)	2 plates	C2002
96-Well Block (capacity 2 ml/well)	2 plates	P1001-2
Elution Plate (capacity 0.35 ml/well)	2 plates	C2003
ZR-96 MagStand	1	P1005

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