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INSTRUCTION MANUAL

Direct-zol™-96 MagBead RNA

Catalog Nos. **R2100, R2101, R2102, R2103, R2104** & **R2105**

Highlights

- High-throughput, magnetic bead-based purification of high-quality (DNA-free) total RNA ***directly*** from TRIzol®, TRI Reagent® and other acid-guanidinium-phenol based reagents (RNAzol®, QIAzol®, TriPure™, TriSure™, *etc.*).
- Bypasses phase separation and precipitation procedures, for non-biased recovery of miRNA.

Contents

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

Ver. 1.0.6

ZYMO RESEARCH CORP.

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

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.

Product Contents

Direct-zol™-96 MagBead RNA Kit Size (Preps)	**R2100** (2x 96)	**R2101*** (2x 96)	**R2102** (4x 96)	**R2103*** (4x 96)	**R2104** (8x 96)	**R2105*** (8x 96)
TRI Reagent®	-	200 ml	-	2x 200 ml	-	4x 200 ml
MagBinding Beads	6 ml	6 ml	12 ml	12 ml	24 ml	24 ml
Direct-zol™ Binding Buffer[1] (concentrate)	10 ml	10 ml	20 ml	20 ml	2x 20 ml	2x 20 ml
Direct-zol™ MagBead PreWash	200 ml	200 ml	2x 200 ml	2x 200 ml	4x 200 ml	4x 200 ml
DNase I[2]	4	4	8	8	16	16
DNA Digestion Buffer	4 ml	4 ml	4 ml	4 ml	2x 4 ml	2x 4 ml
DNase/RNase-Free Water	30 ml	30 ml	2x 30 ml	2x 30 ml	4x 30 ml	4x 30 ml
Collection Plate	2	2	4	4	8	8
Elution Plate	2	2	4	4	8	8
96-Well Plate Cover Foil	2	2	4	4	8	8
Instruction Manual	1	1	1	1	1	1

Storage Temperature - Store all kit components (*i.e.,* buffers, plates) at room temperature.
TRI Reagent® is provided only with catalog numbers **R2101, R2103** & **R2105**.
[1] **Upon arrival**, add 40 or 80 ml ethanol (95-100%) to the 10 or 20 ml **Direct-zol™ Binding Buffer** concentrate, respectively.
[2] Prior to use, reconstitute the lyophilized **DNase I**. See instructions (page 3).

Specifications

- **Sample Sources** – Any sample stored and preserved in TRI Reagent®, TRIzol® or similar*. (animal cells, tissue, biological fluids (*e.g.,* blood, plasma, serum, CSF), and *in vitro* processed RNA (*e.g.,* transcription products, DNase-treated or labeled RNA)).
- **Sample inactivation** – TRI Reagent® (provided with R2101, R2103, and R2105 only) inhibits RNase activity and inactivates viruses and other infectious agents.
- **RNA Size** – RNAs ≥17 nucleotides.
- **RNA Purity** – A_{260}/A_{280} >1.8, A_{260}/A_{230} >1.8. Complete removal of DNA is performed with DNase I digestion (page 4).
- **RNA Recovery** – The RNA binding capacity is up to ~10 µg (per 20 µl **MagBinding Beads**).
- **RNA Storage** – RNA eluted with the **DNase/RNase-Free Water** (provided) can be stored at ≤-70ºC. The addition of RNase inhibitors (optional) is highly recommended for prolonged storage.
- **Equipment Needed** – Magnetic stand (manual processing) or a strong-field 96-well magnetic stand (*i.e.,* **ZR-96 MagStand**, P1005) and a liquid handler with heating element and plate shaker (automated processing).

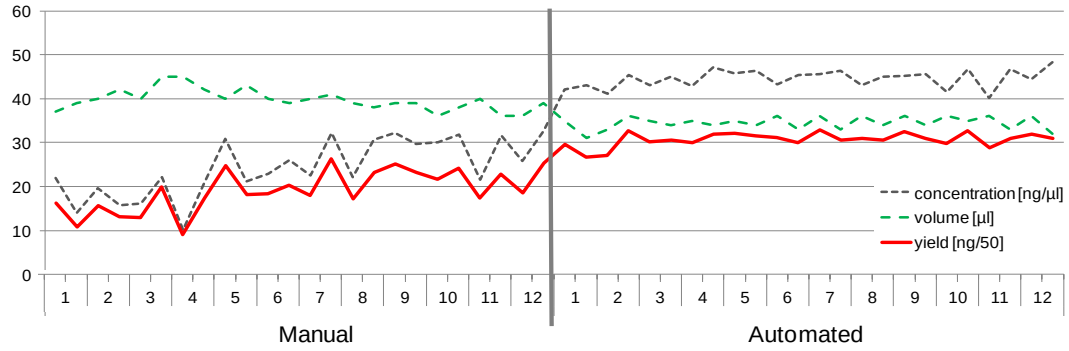
This product is for research use only and not intended for use in diagnostic procedures.
Follow applicable federal, state, and local regulations for phenol waste disposal.

Product Description

The **Direct-zol™-96 MagBead RNA** provides a high-throughput, magnetic bead-based purification 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 and non-coding RNAs (17-200 nt) is effectively isolated from a variety of sample sources (cells, tissues, serum, plasma, blood, biological liquids, *etc.*).

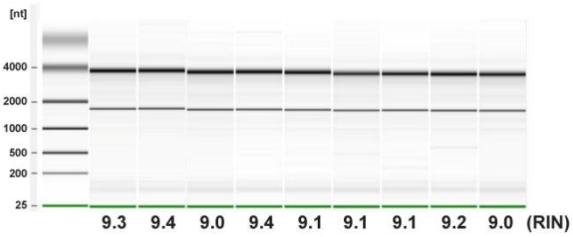
The procedure is easy: simply add **Direct-zol™ Binding Buffer** and **MagBinding Beads** to a sample in TRI Reagent®, wash and elute the RNA. No phase separation, precipitation, or post-purification steps are necessary. The result is broad range purification of small and large RNAs suitable for subsequent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

Reproducible Sample Processing



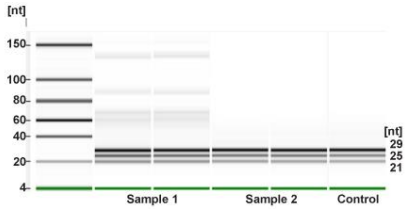
Comparison between manual and automated (Freedom EVO®, Tecan) sample processing with the **Direct-zol™-96 MagBead RNA** across a 96-well plate. RNA was purified from human epithelial cells (5x 10^5/well).

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 **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 & R2105 supplied with TRI Reagent®.

Catalog Nos. R2100, R2102 & R2104 do not include TRI Reagent®.

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

Unless specified otherwise, all steps should be performed at room temperature.

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.

Buffer Preparation

- ✓ Upon arrival, add 40 or 80 ml ethanol (95-100%) to the 10 or 20 ml **Direct-zol™ Binding Buffer** concentrate, respectively.
- ✓ 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 **DNase I** with **DNase/RNase-Free Water** (table above), transfer into an RNase-free tube (e.g., 15 ml conical tube; not provided) and mix by inversion.

   At this point, aliquots can be stored frozen at -20°C.
2. Add **DNA Digestion Buffer** (table above) and mix by inversion, then place on ice until ready to use.
3. Add 50 µl **DNase I Reaction Mix** per sample/well during RNA Purification (page 4, step 7).

Notes:

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

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. For detailed processing information, refer to the TRI Reagent® product manual (or manufacturer's instructions for the reagent used).

(I) Sample Preparation

All centrifugation steps should be performed at 10,000-16,000 x *g* for 1 minute.

1. To lyse a sample, resuspend cells or homogenize tissue in an appropriate volume (see table below) of TRI Reagent®, TRIzol® or similar[1] acid-guanidinium-phenol reagents.

Animal	Tissue	Biological Fluids	Add TRI Reagent®
≤ 10^6	≤ 5 mg	≤ 50 µl	150 µl

2. To remove particulate debris, centrifuge and transfer the supernatant (up to 150 µl/well) into a 96-well **Collection Plate**[2] (or an RNase-free tube; not provided). Proceed to RNA Purification (page 4).

[2] This protocol can be performed using a 96-well **Collection Plate** (C2002; capacity is up to 1.2 ml/well), **96-Well Block** (P1001; capacity is up to 2 ml/well) or an RNase-free tube (not provided).

(II) RNA Purification

1. Add 150 µl **Direct-zol™ Binding Buffer**[1] to 150 µl cleared sample lysate in TRI Reagent® or similar[2], and mix well[3].
2. Add 20 µl **MagBinding Beads** and mix well for 10 minutes.

   Important: MagBinding Beads settle quickly, ensure that beads are kept in suspension while dispensing.
3. Transfer the plate (or tube) to the magnetic stand[4] (sold separately) until beads have pelleted, then aspirate[5] and discard the cleared supernatant.
4. Add 500 µl ethanol (95-100%) and mix well.
5. Transfer the plate (or tube) to the magnetic stand until beads have pelleted, then aspirate and discard the cleared supernatant.
6. Repeat the wash (steps 4-5), two more times.
7. Add 50 µl **DNase I Reaction Mix** and mix for 10 minutes.
8. Add 500 µl **Direct-zol™ MagBead PreWash** and mix well for 10 minutes.
9. Transfer the plate (or tube) to the magnetic stand until beads have pelleted, then aspirate and discard the cleared supernatant.
10. Repeat the prewash and mix well for 1 minute (steps 8-9).

    Set a heating element to 55°C. Depending on the time necessary for the element to reach temperature, this can be performed at any time prior to step 14.
11. Add 500 µl ethanol (95-100%) and mix well.
12. Transfer the plate (or tube) to the magnetic stand until beads have pelleted, then aspirate and discard the cleared supernatant.
13. Repeat the wash (steps 11-12), two more times.
14. To dry the beads, transfer the plate (or tube) to a heated element and incubate at 55°C for 1 hour[6].
15. To elute RNA from the beads, add 50 µl of **DNase/RNase-Free Water** and mix well for 5 minutes.
16. Transfer the plate (or tube) to the magnetic stand until beads have pelleted, then aspirate and dispense the eluted RNA to an **Elution Plate** (or new tube).

    The eluted RNA can be used immediately or stored frozen.

    Use the **Cover Foil** to prevent evaporation.

Perform all steps at room temperature.

Notes:

[1] Upon arrival, add ethanol to the buffer concentrate (Buffer Preparation, page 3).

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

[3] For all buffer additions, **mix well** by pipetting up and down several times and/or (if available) by vortexing at ~1,300 rpm.

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

[5] 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.

[6] Beads will change in appearance from glossy black when still wet to a dull brown when fully dry.

Notes:

[1] *e.g.,* Tecan Freedom EVO®

Automation Scripts

The **Direct-zol™-96 MagBead RNA** is compatible with automated platforms[1]. For automation scripts and related technical support, email tech@zymoresearch.com. In the subject line, please include "Automation Scripts", instrument used and the product catalog number.

Ordering Information

Product Description	Catalog No.	Kit Size
Direct-zol™-96 MagBead RNA (TRI Reagent® not included)	R2100 R2102 R2104	2x 96 preps. 4x 96 preps. 8x 96 preps.
Direct-zol™-96 MagBead RNA (supplied with TRI Reagent®)	R2101 R2103 R2105	2x 96 preps. 4x 96 preps. 8x 96 preps.

Individual Kit Components	Catalog No.	Amount
TRI Reagent®	R2050-1-50 R2050-1-200	50 ml 200 ml
Direct-zol™ Binding Buffer (concentrate)	R2100-1-10 R2100-1-20	10 ml 20 ml
Direct-zol™ MagBead PreWash	R2100-2-200	200 ml
DNase/RNase-Free Water	W1001-1 W1001-4 W1001-6 W1001-10 W1001-30	1 ml 4 ml 6 ml 10 ml 30 ml
DNase I (lyophilized) (250 U supplied with DNA Digestion Buffer, 4 ml)	E1010	1 set
MagBinding Beads	D4100-2-6 D4100-2-12 D4100-2-24	6 ml 12 ml 24 ml
96-Well Plate Cover Foil	C2007-2 C2007-4 C2007-8	2 4 8
Collection Plate	C2002	2 plates
Elution Plate	C2003	2 plates
ZR-96 MagStand	P1005	1

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