ZYMO RESEARCH

RNA Purification Made Simple™

RNA Clean & Concentrator™ MagBead

Clean-up RNA from any sample

Highlights

- High-throughput, magnetic-bead based clean-up of total RNA (including small/microRNAs) from any enzymatic reaction, aqueous phase following TRIzol extraction, in vitro transcription products, etc.
- Ultra-pure RNA is ready for Next-Gen Sequencing, RT-qPCR, etc. *DNase I is included.*

Catalog Numbers:
R1081, R1082 (supplied with DNase I Set)

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

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Table of Contents

Product Contents

RNA Clean & Concentrator™ MagBead	R1081 (96 prep)	R1082 (96 prep)
RNA MagBead Binding Buffer	15 ml (x2)	15 ml (x2)
MagBinding Beads	6 ml	6 ml
RNA Prep Buffer	100 ml	100 ml
DNase I[1] (lyophilized)	-	250 U (x2)
DNA Digestion Buffer	-	4 ml
DNase/RNase-Free Water	10 ml	10 ml
Instruction Manual	1 pc	1 pc

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature.
Before use:
1 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**

Specifications

- **Sample Sources –** Enzymatic reactions (e.g., DNase I treated RNA), the aqueous phase following TRIzol®/chloroform or similar[1] extraction, in vitro transcriptions, etc.

- **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 total RNA per 15 µl **MagBinding Beads**.

- **Elution Volume –** ≥ 15 µl **DNase/RNase-Free Water**.

- **Materials Needed** (user provided) **–** Isopropanol (100%), ethanol (95-100%), magnetic stand, nuclease-free tubes or 96-well plates
- **Recommended Materials** (materials sold 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, 4 pack), DNase/RNase-Free Tubes (C2001; 100 pack).

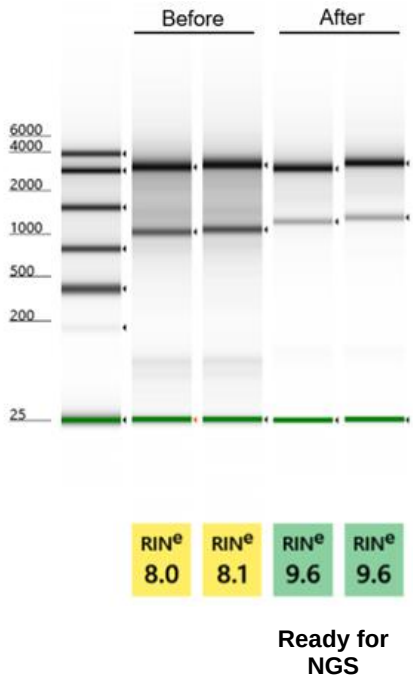
- **Chemical Tolerance –** ≤5% Triton X-100, ≤5% Tween-20, ≤5% Sarkosyl, ≤ 0.1% SDS. Also compatible with ≤90% Sucrose, ≤90% Formamide, and ≤2% Formaldehyde.

1 TRI Reagent®, RNAzol®, QIAzol®, TriPure™, TriSure™, and all other acid guanidinium-phenol reagents.

Product Description

The **RNA Clean & Concentrator™ MagBead** kit provides a high-throughput, magnetic-bead based clean-up method of any RNA sample such as DNase I treated RNA, in-vitro transcription products and the aqueous phase of TRIzol/chloroform or similar extraction. High-quality RNA is ready for Next-Gen sequencing, RT-qPCR and other downstream applications.

The procedure is simple: Add binding buffer and **MagBinding Beads** to your sample, then wash and elute ultra-pure RNA. The unique single-buffer system and magnetic bead technology can be used on an automated platform (or manually) for high-throughput processing.



Clean-up any RNA sample for NGS, RT-qPCR, etc.
Profile of total RNA before and after clean-up with the **RNA Clean & Concentrator™ MagBead** kit (Agilent 2200 TapeStation).

Protocol

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

(I) Buffer Preparation

- ✓ Prior to use, 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**
  #E1011-A (1500 U), add 1,500 µl **water**

(II) Total RNA Clean-up

- ✓ RNA species ≥ 17 nt will be recovered.
- ✓ For all buffer additions and incubation steps, **mix well** by pipetting up and down and/or by shaking (vortexing) at ~1,000 rpm for 3 minutes. Optimization may be required.
- ✓ For DNA-free RNA (optional), perform **DNase I** treatment before clean-up (page 6).

1. Add 150 µl of **RNA MagBinding Buffer** (3 volumes) to 50 µl sample[1] and mix well.
2. Add 15 µl **MagBinding Beads** and mix well.

   Important: **MagBinding Beads** settle quickly, ensure that beads are kept in suspension while dispensing.
3. Add 250 µl of 100% isopropanol (1.25 volumes) and mix well for 15 minutes.
4. Transfer the plate/tube to the magnetic stand[2] (sold separately) until beads have pelleted, then aspirate[3] and discard the cleared supernatant.
5. Add 500 µl of **RNA Prep Buffer** and mix well. Pellet the beads[2,3] and discard the supernatant.
6. Add 500 µl of ethanol (95-100%) and mix well. Pellet the beads[2,3] and discard the supernatant.
7. Add 500 µl of ethanol (95-100%) and mix well.
8. Transfer the sample (beads and liquid) to a new plate/tube. Pellet the beads[2,3] and discard the supernatant.
9. Dry the beads at room temperature for 10 minutes or until dry[4].
10. To elute DNA/RNA from the beads, add 15 µl **DNase/RNase-Free Water** and mix well for 5 minutes.
11. Transfer the plate/tube to the magnetic stand 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 To minimize pipetting error, adjust the sample volume to 50 µl (minimum).
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 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

RNA Clean & Concentrator™ MagBead (R1081/R1082) 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.

DNase I Treatment

Before RNA Clean-up

For each sample to be treated, prepare 50 µl **DNase I Reaction Mix** in an RNase-free tube (not provided) and mix by gentle inversion. Then incubate at room temperature (20-30°C) for 15 minutes and proceed with the RNA Clean-up protocol, page 5, step 1.

DNase I Reaction Mix	
RNA sample (≤ 10 µg; volume adjusted with water or TE buffer)	40 µl
DNase I (reconstituted; 1 U/ul)[1]	5 µl
DNA Digestion Buffer	5 µl

RNA clean-up from aqueous phase after TRIzol®/chloroform extraction

Following TRIzol®/chloroform or similar* extraction, carefully transfer the upper aqueous phase into an RNase-free tube (not provided). Add an equal volume of **RNA MagBinding Buffer** to 1 volume sample[2] (1:1) and mix well. Then proceed to page 5, step 2 of the RNA Clean-up protocol.

RNA clean-up from samples in DNA/RNA Shield™

1. If frozen, thaw samples to room temperature (20-30°C) and centrifuge debris (if any). Transfer the cleared sample into an RNase-free tube (not provided).
2. Add 1 volume of **RNA MagBinding Buffer** to 1 volume of the **DNA/RNA Shield™** sample[2] (1:1) and mix well.
3. Continue with the RNA Clean-up protocol, page 5, step 2.

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

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.

2 To minimize pipetting error, adjust the sample volume to 50 µl (minimum).

Purification of Small and Large RNAs into Separate Fractions

1. Add 150 µl of **RNA MagBinding Buffer** (3 volumes) to 50 µl sample[1] and mix well.
2. Add 15 µl **MagBinding Beads** and mix well.
3. Add 100 µl of 95-100% ethanol (0.5 volume) and mix well for 15 minutes.
4. Transfer the plate/tube to the magnetic stand[2] (sold separately) until beads have pelleted. Aspirate[3] and save the supernatant!

5. **Small RNAs (17-200 nt)**
   are in the supernatant

   a. Add 15 µl **MagBinding Beads** and mix well.

      Important: **MagBinding Beads** settle quickly, ensure that beads are kept in suspension while dispensing.

   b. Add 300 µl of 100% isopropanol (equal volume) and mix well for 15 minutes.

   c. Transfer the plate/tube to the magnetic stand[2] (sold separately) until beads have pelleted, then aspirate[3] and discard the cleared supernatant.

   d. Proceed to page 5, step 5 of the RNA Clean-up protocol.

5. **Large RNAs (> 200 nt)**
   are bound to the beads

   a. Proceed to page 5, step 5 of the RNA Clean-up protocol.

cDNA Clean-Up following Reverse Transcription (RT)

The **RNA Clean & Concentrator** can be used to effectively clean and concentrate first-strand cDNA following reverse transcription (RT) and hydrolysis. The **RNA Binding Buffer** will neutralize the hydrolysis reaction and the recovered cDNA may be used directly for microarray analysis, etc.

Hydrolysis Reaction: To each 30-50 µl RT reaction, add 10 µl 0.5 M EDTA and 10 µl 1 M NaOH. Then mix and incubate at 65°C for 15 minutes. Proceed to the Total RNA Clean-Up protocol, page 5.

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1 To minimize pipetting error, adjust the sample volume to 50 µl (minimum).
2 Use a strong-field magnetic stand or separator (e.g., ZR-96 MagStand, P1005; sold separately) to pellet beads.
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.

Ordering Information

Product Description	Catalog No.	Size
RNA Clean & Concentrator™ MagBead	R1081	96 preps.
RNA Clean & Concentrator™ MagBead (supplied with DNase I Set)	R1082	96 preps.

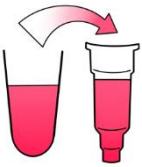
Individual Kit Components	Catalog No.	Amount
RNA MagBead Binding Buffer	R1081-1	15 ml
MagBinding Beads	D4100-2-3 D4100-2-6 D4100-2-12	3 ml 6 ml 12 ml
RNA Prep Buffer	R1060-2-10 R1060-2-25 R1060-2-100	10 ml 25 ml 100 ml
DNase/RNase-Free Water	W1001-10 W1001-30	10 ml 30 ml
DNase I (lyophilized) (250 U supplied with DNA Digestion Buffer, 4 ml)	E1010	1
ZR-96 MagStand	P1005	1
Collection Plate (capacity 1.2 ml/well)	C2002	2
96-Well Block (capacity 2 ml/well)	P1001-2	2
Elution Plate (capacity 0.35 ml/well)	C2003	2
96-Well Plate Cover Foil	C2007-2 C2007-4	2 4

Complete Your Workflow

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

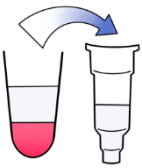
ZR BashingBead Lysis Tubes	
2.0 mm beads #S6003	For plant/animal tissue
0.1 + 0.5 mm beads #S6012	For microbes
0.1 + 2.0 mm beads #S6014	For microbes in tissue/insects

✓ 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 purity (A_{260}/A_{230} nm, A_{260}/A_{280} nm)	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	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 prior to the RNA Clean-up protocol, page 6 - - .

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

Notes

Notes

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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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