ZYMO RESEARCH

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INSTRUCTION MANUAL

RNA Clean & Concentrator™ MagBead

Catalog Nos. **R1081**
R1082 (supplied with DNase I Set)

Highlights

- **High-throughput:** Magnetic bead-based clean-up of total RNA (including small/micro RNAs) from any enzymatic reaction and the aqueous phase following TRIzol® extractions.
- **Ultra-Pure:** High-quality RNA ready for Next-Gen sequencing, RT-qPCR, hybridization etc. *DNase I included.*

Contents

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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.

Product Contents

RNA Clean & Concentrator™ MagBead (Kit Size)	**R1081** (96 Preps)	**R1082** (96 Preps)	**Storage Temperature**
RNA MagBead Binding Buffer	2 x 15 ml	2 x 15 ml	Room Temp.
MagBinding Beads	6 ml	6 ml	Room Temp.
RNA Prep Buffer	100 ml	100 ml	Room Temp.
DNase I[1] (lyophilized)	-	2 x 250 U	Room Temp. (-20°C; reconstituted)
DNA Digestion Buffer	-	4 ml	Room Temp.
DNase/RNase-Free Water	10 ml	10 ml	Room Temp.
Instruction Manual	1	1	-

Note - 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 maximal performance and reliability.

[1] Before use, reconstitute each vial of lyophilized **DNase I** (E1009-A) with 275 µl **DNase/RNase-Free Water**, mix gently and store frozen aliquots.

Notes:

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

Specifications

- **Sample Sources**: DNase I treated RNA, in vitro transcription products or the aqueous phase following TRIzol®/chloroform or similar* extraction (page 4).
- **Binding Capacity:** 10 µg total RNA per 15 µl **MagBinding Beads**.
- **Purity:** RNA is ready for Next-Gen Sequencing, RT-qPCR, etc. DNase I included (R1082 only).
- **RNA Storage:** RNA eluted with **DNase/RNase-Free Water** can be stored frozen. The addition of RNase inhibitors is optional but highly recommended for prolonged storage.
- **User Supplied:** Isopropanol (100%), ethanol (95-100%), or strong-field magnetic stand
- **Recommended Materials** (available separately): 96-well Collection Plate (C2002; capacity is up to 1.2 ml/well), 96-Well Block (P1001; capacity is up to 2 ml/well), Elution Plate (C2003), Cover Foil (C2007), and ZR-96 MagStand (P1005).

Automation Scripts

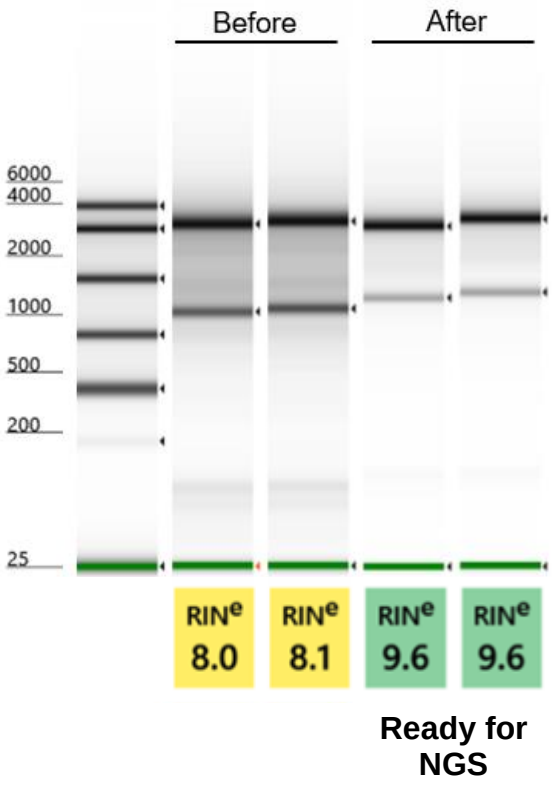
RNA Clean & Concentrator™ MagBead (R1081/R1082) is compatible with automated platforms. For automation scripts and related technical support, email automation@zymoresearch.com. In the subject line, please include "Automation Scripts", instrument used and the product catalog number.

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.

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 following 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 manually or on an automated platform for high-throughput processing.



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

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

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

Notes:

[1] Prior to use, reconstitute the lyophilized **DNase I** (E1009-A) with 275 µl **DNase/RNase-Free Water**, mix gently and store frozen aliquots.

* 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).

[3] Use a strong-field magnetic stand or separator (e.g., **ZR-96 MagStand**, P1005) until beads have pelleted.

[4] Some beads will adhere to the sides of the well. When removing the supernatant, aspirate slowly to allow these beads to be pulled to the magnet as the liquid level is lowered.

[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 (25-55ºC).

Protocol

The following procedure should be performed at room temperature (15-30°C), unless specified.
For DNA-free RNA, perform DNase I treatment prior to clean-up protocol.

DNase I treatment before clean-up[1] (optional)

(1) For each sample, prepare DNase I reaction mix in an RNase-free tube. Mix well by gentle inversion:

RNA sample (≤ 10 µg; volume adjusted with water or TE buffer	40 µl
DNase I (reconstituted; 1 U/µl)*	5 µl
DNA Digestion Buffer	5 µl
	50 µl

(2) Incubate at room temperature (20-30°C) for 15 minutes, then proceed with RNA Clean-Up, below.

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.

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

   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[3] (sold separately) until beads have pelleted, then aspirate[4] and discard the cleared supernatant.
5. Add 500 µl of **RNA Prep Buffer** and mix well. Pellet the beads[3,4] and discard the supernatant.
6. Add 500 µl of ethanol (95-100%) and mix well. Pellet the beads[3,4] 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[3,4] and discard the supernatant.
9. Dry the beads at room temperature for 10 minutes or until dry[5].
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.
    For assistance with automating/scripting for this workflow onto your platform or device, contact one of our automation specialists at automation@zymoresearch.com

Appendices

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

Following TRIzol®/TRI Reagent® or similar* extraction, carefully transfer the upper aqueous phase into an RNase-free tube. Add 50 µl of **RNA MagBinding Buffer** (1 volume) to 50 µl sample[1] and mix well. Then proceed to page 3, step 2 of the RNA Clean-Up protocol.

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 3, step 5 of the RNA Clean-Up protocol.

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

   a. Proceed to page 3, step 5 of the RNA Clean-Up protocol.

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

Notes:

[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) until beads have pelleted.

[3] Some beads will adhere to the sides of the well. 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	Kit Size	Catalog No.
RNA Clean & Concentrator™ MagBead	96 Preps	R1081
RNA Clean & Concentrator™ MagBead (supplied with DNase I Set)	96 Preps	R1082

For Individual Sale	Amount	Catalog No.
RNA MagBead Binding Buffer	15 ml	R1081-1-15
MagBinding Beads	3 ml 6 ml 8 ml 12 ml 16 ml 24 ml	D4100-2-3 D4100-2-6 D4100-2-8 D4100-2-12 D4100-2-16 D4100-2-24
RNA Prep Buffer	10 ml 25ml 100 ml	R1060-2-10 R1060-2-25 R1060-100
DNase/RNase-Free Water	1 ml 4 ml 6 ml 10 ml	W1001-1 W1001-4 W1001-6 W1001-10
DNase I Set DNase I (lyophilized; 250 U) & DNA Digestion Buffer (4 ml)	1 Set	E1010

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