ZYMO RESEARCH

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INSTRUCTION MANUAL

Quick-RNA™ MagBead

Catalog Nos. **R2132, R2133**

Highlights

- **Versatile:** High-throughput, magnetic bead-based isolation total RNA (including small/micro RNAs) from any sample including cells, solid tissue, whole blood, biological liquids, FFPE tissue, environmental (plant/seed), swabs (stool, soil, microbial samples), *etc.*
- **NGS-Ready:** High-quality RNA is ready for any downstream application. *DNase I included.*

Contents

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Satisfaction of all Zymo Research products is guaranteed. If you are dissatisfied with this product please contact us.

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.

For assistance, contact us at tech@zymoresearch.com.

Product Contents

***Quick*-RNA™ MagBead** (Kit Size)	**R2132** (96 Preps)	**R2133** (4 x 96 Preps)	**Storage Temperature**
MagBinding Beads	6 ml	24 ml	Room Temp.
DNA/RNA Shield™ (2X concentrate)	25 ml	125 ml	Room Temp.
RNA Lysis Buffer	50 ml	2 x 100 ml	Room Temp.
RNA Prep Buffer	50 ml	2 x 100 ml	Room Temp.
MagBead DNA/RNA Wash 1[1] (concentrate)	2 x 30 ml	2 x 120 ml	Room Temp.
MagBead DNA/RNA Wash 2[2] (concentrate)	2 x 20 ml	2 x 80 ml	Room Temp.
DNase/RNase-Free Water	30 ml	100 ml	Room Temp.
DNase I[3] (lyophilized)	3 x 250 U	2 x 1500 U	Room Temp. (-20°C; reconstituted)
DNA Digestion Buffer	4 ml	4 ml	Room Temp.
Proteinase K[3] (lyophilized) & Storage Buffer	20 mg	4 x 20 mg	Room Temp. (-20°C; reconstituted)
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[1] Before starting, add 20 ml (R2132) or 80 ml (R2133) of isopropanol to the **MagBead DNA/RNA Wash 1** concentrate.

[2] Before starting, add 30 ml (R2132) or 120 ml (R2133) of isopropanol to the **MagBead DNA/RNA Wash 2** concentrate.

[3] Prior to use, reconstitute the lyophilized **DNase** I and lyophilized **Proteinase K**, according to page 3, Reagent Preparation. Store frozen aliquots.

Specifications

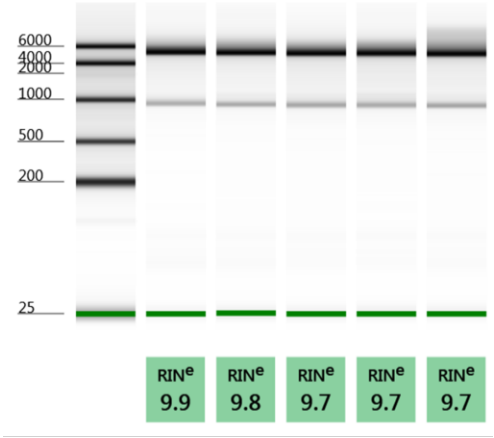
- **Sample Sources** – Any cells, solid tissue, whole blood, biological fluids, FFPE tissue, environmental (plant/seed), swabs (stool, soil, microbial samples), samples stored in DNA/RNA Shield™, *etc.*
- **Sample Preservation** – **DNA/RNA Shield™** lyses cells, inactivates nucleases and infectious agents and is ideal for safe sample storage and transport at ambient temperatures (page 6).
- **Size Limits** – Total RNA including small/microRNAs ≥17 nt.
- **Purity** – High-quality RNA is ready for Next-Gen Sequencing, RT/PCR, hybridization, etc.
- **Binding Capacity** – 15 µg total RNA per 30 µl **MagBinding Beads**.
- **Storage** – RNA eluted with **DNase/RNase-Free Water** (provided) can be stored frozen. The addition of RNase inhibitors is highly recommended for prolonged storage.
- **Equipment Needed** – Magnetic stand or separator, heat block, liquid handler or robotic sample processer (user provided).
- **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), 96-well Elution Plate (C2003), Cover Foil (C2007), ZR-96 MagStand (P1005).

Product Description

The ***Quick*-RNA™ MagBead** kit provides a high-throughput, magnetic bead-based purification of high-quality total RNA (including small/microRNAs) from any sample source (e.g., cells, solid tissue, whole blood, biological fluids, FFPE tissue, environmental (plant/seed), swabs (stool, soil, microbial samples)), samples stored in **DNA/RNA Shield™**, *etc.* The provided DNA/RNA Shield™ inactivates infectious agents and is ideal for sample storage at ambient temperatures. Total RNA is eluted into ≥50 µl of **DNase/RNase-Free Water** and is ready for any downstream application including Next-Gen Sequencing, RT/PCR, hybridization, *etc.*

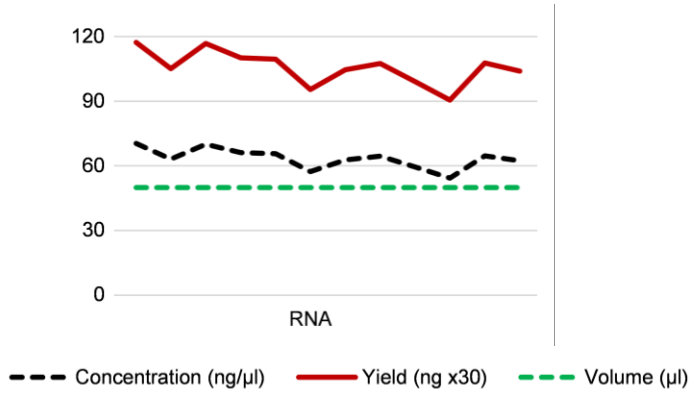
For **Assistance**, please contact Zymo Research Technical Support at 1-888-882-9682 or e-mail tech@zymoresearch.com.

High-Quality RNA



Total RNA quality assessed using Agilent 2200 TapeStation. RNA was purified from HeLa cells using the ***Quick*-RNA™ MagBead** kit.

Reproducible Sample Processing



Concentration, yield, and elution volume across replicate samples extracted with the ***Quick*-RNA™ MagBead** are reproducible and consistent. RNA was purified from HeLa cells (2.5 x 10^5/well).

The lyophilized **Proteinase K** and **DNase I** are stable as shipped.

*Unit definition – one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A260 units/min/ml of reaction mixture at 25°C.

Reagent Preparation

- ✓ Add 20 ml (R2132) or 80 ml (R2133) isopropanol to the **MagBead DNA/RNA Wash 1** concentrate.
- ✓ Add 30 ml (R2132) or 120 ml (R2133) isopropanol to the **MagBead DNA/RNA Wash 2** concentrate.
- ✓ Add 1.2 ml **Proteinase K Storage Buffer** per vial to reconstitute the lyophilized **Proteinase K** at 20 mg/ml. Vortex to dissolve. Store frozen aliquots.
- ✓ Prepare **DNase I Reaction Mix** (according to the example below; scale as needed):

Prep Size	DNase I (lyophilized)	DNase/RNase-Free Water	DNA Digestion Buffer
96 preps	3 x 250 U	6.75 ml	0.75 ml
4 x 96 preps	2 x 1500 U	27 ml	3 ml

a. 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. Store frozen aliquots.

b. Add **DNA Digestion Buffer** to the reconstituted DNase I (table above) and mix by inversion, then place on ice until ready to use. Add 50 µl **DNase I Reaction Mix** per sample during RNA Purification, page 5.

Protocol

The isolation consists of: (I) Sample Preparation and (II) RNA Purification

(I) Sample Preparation

All centrifugation steps should be performed at 10,000 - 16,000 *x g* for 30 seconds, unless specified.
All steps should be performed at room temperature (20-30ºC), unless specified.

Cells

1. Pellet up to 10^6 mammalian cells (≤500 *x g* for 1 minute), remove the supernatant and resuspend the cell pellet in 200 µl **DNA/RNA Shield**™ (1X)[1]. Proceed to RNA Purification, page 5.

Solid Tissue & Blood Cells (PBMCs, WBCs)

1. Add ≥200 µl **DNA/RNA Shield**™ (1X)[1] to a solid tissue sample (≤5 mg) and mechanically homogenize[2,3]. Centrifuge to pellet debris and transfer 200 µl supernatant to a new tube. For blood cells, buffy coat and pelleted PAXgene™ samples (≤1 ml blood) resuspend in 200 µl **DNA/RNA Shield**™ (1X)[1].
2. Add 10 µl **Proteinase K** for every 200 µl sample. Mix and incubate at room temperature (20-30ºC) for 30 minutes.
3. Proceed to RNA Purification, page 5.

Urine[4]

1. Generate pellet from up to 40 ml urine by adding 70 µl **Urine Conditioning Buffer** for every 1 ml of urine and mix by vortexing. Centrifuge at 3,000 *x g* for 15 minutes. Discard the supernatant and leave up to 50 µl pellet.
2. Add 150 µl **DNA/RNA Shield**™ (1X)[1] and resuspend the pellet by pipetting.
3. Add 10 µl **Proteinase K**. Mix and incubate at room temperature (20-30ºC) for 30 minutes.
4. Proceed to RNA Purification, page 5.

Notes:

[1] To prepare 1X solution, mix equal amounts of the supplied 2X concentrate with nuclease-free water (not provided).

2 ZR-96 BashingBead™ Lysis Rack (2.0 mm) (S6002-96-2) and ZR BashingBead™ Lysis Tubes (2.0 mm) (S6003-50) are sold separately.

3 Processing time will vary based on sample input and bead beater. For high-speed homogenizers (e.g. Precellys, FastPrep®), process for ≤ 5 minutes. For low-speed homogenizers (e.g., Disruptor Genie), process for ≥ 10 minutes.

[4] Warm up urine sample at 37˚C for 5-10 minutes if the urine is visually cloudy (salt precipitation). Samples containing bacterial contamination will not be clear.

After adding Urine Conditioning Buffer (sold separately; D3061-1-140), urine can be stored for up to 1 month at ambient temperature. Prior to processing, mix the sample thoroughly by vortexing.

(I) Sample Preparation (continued)

Whole Blood[1] (Mammalian)

1. Add 200 µl **DNA/RNA Shield™** (2X concentrate) to each 200 µl sample and mix thoroughly.
2. For every 400 µl of reagent/sample mixture, add 10 µl **Proteinase K** and mix thoroughly. Incubate at room temperature (20-30ºC) for 30 minutes.
3. Add 1 volume of isopropanol and mix well.
4. Transfer 800 µl of the sample mixture into a new tube and proceed to RNA Purification, page 5, step 3.

FFPE Tissue

1. Remove (trim) excess paraffin wax from ≤5 mg FFPE tissue and transfer into a new tube.
2. Add 400 µl **Deparaffinization Solution**[2] to the sample. Incubate at 55ºC for 1 minute. Vortex briefly. Remove the **Deparaffinization Solution**.
3. Add 95 µl **DNase/RNase-Free Water**, 95 µl **2X Digestion Buffer**[2], and 10 µl **Proteinase K**.
4. Incubate at 55ºC for 1 hour. Incubate at 94ºC for 20 minutes to de-crosslink the sample.
5. Centrifuge to remove insoluble debris and transfer 200 µl supernatant to a new tube.
6. Proceed to RNA Purification, page 5.

Environmental (Plant/Seed, *etc.*)

1. Add up to 50 mg plant material and 750 µl **DNA/RNA Shield™** (1X)[3] to a **lysis tube/rack**[4].
2. Secure in a bead beater fitted with the appropriate holder assembly for your bead beating module and process at maximum speed[5] for 1 minute.
3. Add 10 µl **Proteinase K**. Mix and incubate at room temperature (20-30ºC) for 30 minutes.
4. Centrifuge to pellet debris and transfer 200 µl supernatant to a new tube.
5. Proceed to RNA Purification, page 5.

Swabs (Stool, Soil, Microbial samples, *etc.*)

1. Add 750 µl **DNA/RNA Shield™** (1X)[3] to a swab sample (or up to 50 mg stool or soil) and mix by vortexing. Centrifuge to pellet debris and transfer 200 µl supernatant to a new tube.

   Optional: To achieve unbiased lysis of different organisms (including hard-to-lyse microbes), add sample in **DNA/RNA Shield™** to a **lysis tube/rack**[6]. Secure in a bead beater fitted with the appropriate holder assembly for your bead beating module and process at maximum speed[5] for 5 minutes.
2. Add 10 µl **Proteinase K**. Mix and incubate at room temperature (20-30ºC) for 30 minutes.
3. Proceed to RNA Purification, page 5.

Notes:

[1] Compatible with commonly used anticoagulants (*i.e.,* EDTA, citrate, heparin).

[2] Deparaffinization Solution (D3067-1-20) and 2X Digestion Buffer (D3050-1-20) are sold separately.

[3] To prepare 1X solution, mix equal amounts of the supplied 2X concentrate with nuclease-free water (not provided).

[4] ZR-96 BashingBead™ Lysis Rack (2.0 mm) (S6002-96-2) and ZR BashingBead™ Lysis Tubes (2.0 mm) (S6003-50) are sold separately.

[5] Processing time will vary based on sample input and bead beater. For high-speed homogenizers (*e.g.* Precellys, FastPrep®), process for ≤ 5 minutes. For low-speed homogenizers (*e.g.,* Disruptor Genie), process for ≥ 10 minutes.

[6] ZR-96 BashingBead™ Lysis Rack (0.1 & 0.5 mm) (S6002-96-3) and ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm) (S6012-50) are sold separately.

Perform all steps at **room temperature**, unless specified.

Notes:

[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. 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 (25-55ºC).

(II) RNA Purification

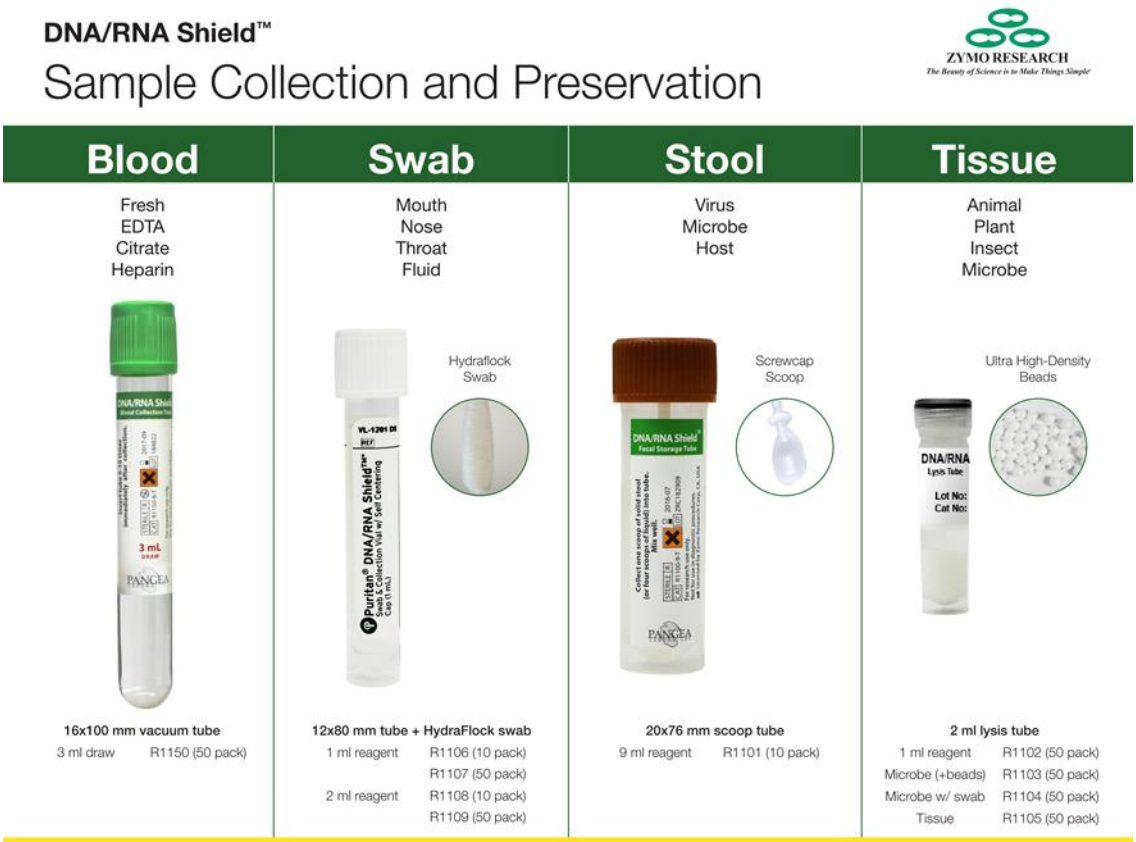
1. Add 200 µl (1 volume) **RNA Lysis Buffer** to 200 µl sample and mix well[1].
2. Add 400 µl ethanol (95-100%) to the sample and mix well[1].
3. Add 30 µl **MagBinding Beads** and mix well[1] for 20 minutes.

   Important: **MagBinding Beads** settle quickly, ensure that beads are kept in suspension while dispensing.
4. Transfer the plate/tube to the magnetic stand[2] until beads have pelleted, then aspirate[3] and discard the cleared supernatant.
5. Add 500 µl **MagBead DNA/RNA Wash 1** and mix well[1]. Pellet the beads[2,3] and discard the supernatant.
6. Add 500 µl **MagBead DNA/RNA Wash 2** and mix well[1]. Pellet the beads[2,3] and discard the supernatant.
7. Add 500 µl ethanol (95-100%) and mix well[1]. Pellet the beads[2,3] and discard the supernatant.
8. Repeat step 7.
9. **DNase I** treatment (optional)
   - (D1) Add 50 µl **DNase I Reaction Mix** and mix gently for 10 minutes.
   - (D2) Add 500 µl **DNA/RNA Prep Buffer** and mix well[1] for 10 minutes. Pellet the beads[2,3] and discard the supernatant.
   - (D3) Repeat steps 7-8.
10. Dry the beads for 10 minutes or until dry[4].
11. To elute RNA from the beads, add 50 µl **DNase/RNase-Free Water** and mix well[1] for 5 minutes.
12. 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.

For assistance with automating/scripting this workflow onto your platform or device, contact our automation specialists at automation@zymoresearch.com.

Appendix A: Compatible DNA/RNA Shield™ Collection Devices



Appendix B: Sample Preservation in DNA/RNA Shield™

DNA/RNA Shield™ effectively lyses cells, inactivates nucleases and infectious agents and is ideal for sample storage/transport at ambient temperatures prior to nucleic acid purification.

Liquid samples: Mix an equal volume DNA/RNA Shield™ (2X concentrate) and sample.
Solid samples: Submerge sample (not to exceed 10% (v/v or w/v)) in DNA/RNA Shield (1X).

Mix well/homogenize sample prior to storage.

Samples in DNA/RNA Shield™ can be stored at ambient temperature (4-30ºC) for a month, 3 days at 37ºC or long term (>1 year) at -20ºC or below.

Automation Scripts

The ***Quick*-RNA™ MagBead** (R2132/R2133) 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.

Troubleshooting

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

Problem	Possible Causes and Suggested Solutions
Low Recovery	
Binding Conditions	• **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 DNA and RNA to be sufficiently bound to 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. • **Magnetic Beads:** MagBinding Beads settle quickly, ensure that beads are kept in suspension while dispensing. • **Adjust Reagent Volumes:** Low recovery may be due to input (high biomass) usually indicative by a viscous, cloudy lysate. Increase or titrate the volume of DNA/RNA Lysis Buffer, magnetic beads and elution volume proportionally.
Elution Parameters	• **Elution Volume:** Elution volume can be increased to ensure adequate recovery (e.g., ≥50 µl). • **Adjust Temperature:** Increase elution temperature to 55ºC. This can improve recovery if beads are "sticky" (for high molecular weight genomic DNA, etc.).
Low Purity ($A_{260/230\ nm}$)	
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.
Drying of Beads	• **Adjust Drying Parameters:** Increase the time for drying the beads (≥10 minutes) at room temperature. Alternatively, a heat block can be used (55°C).
Degraded Nucleic Acids	
Sample Input	• **Sample Preservation:** Check initial sample by establishing kit controls with a known quality and concentration to eliminate artifacts originating from kit to kit variation. Make sure to transfer samples to provided DNA/RNA Shield™ to ensure sample stabilization and minimize degradation effects.
Bead-beating	• **Sample Lysis:** Bead-beating times may need to be optimized to ensure sufficient lysis without compromising sample quality. These exact settings can vary from low to high-speed cell disrupters. Bead-beating will shear genomic DNA to some extent.

Ordering Information

Product Description	Catalog No.	Kit Size
***Quick*-RNA™ MagBead**	R2132 R2133	96 Preps. 4x 96 Preps.

For Individual Sale	Catalog No.	Amount
MagBinding Beads	D4100-2-6 D4100-2-12 D4100-2-24	6 ml 12 ml 24 ml
DNA/RNA Shield™ (2X concentrate)	R1200-25 R1200-125	25 ml 125 ml
RNA Lysis Buffer	R1060-1-50 R1060-1-100 R1060-1-200	50 ml 100 ml 200 ml
RNA Prep Buffer	R1060-2-50 R1060-2-100	50 ml 100 ml
MagBead DNA/RNA Wash 1 (concentrate)	R2130-1-30 R2130-1-120	30 ml 120 ml
MagBead DNA/RNA Wash 2 (concentrate)	R2130-2-20 R2130-2-80	20 ml 80 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 Set (lyophilized) DNase I (250 U) & DNA Digestion Buffer (4 ml)	E1010	1 set
Proteinase K (lyophilized) (supplied with Proteinase K Storage Buffer)	D3001-2-5 D3001-2-20	5 mg set 20 mg set
Collection Plate	C2002	2 plates
Elution Plate	C2003	2 plates
96-Well Plate Cover Foil	C2007-2 C2007-4 C2007-8	2 4 8
ZR-96 MagStand	P1005	1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