



## Quick-DNA/RNA™ MagBead

DNA & RNA from any sample

#### Highlights

- High-throughput, magnetic-bead purification of DNA and total RNA (including small/microRNAs) from any sample including cells, solid tissue, biological liquids, environmental samples, swabs, and any sample in DNA/RNA Shield™
- DNA/RNA Shield™ and Proteinase K are included for unique preservation and lysis technology.
- DNA & RNA are eluted in one elution or into two separate fractions, ready for Next-Gen Sequencing, RT/qPCR, etc. DNase I is included.

Catalog Numbers: R2130, R2131



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







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### **Product Contents**

<i>Quick</i> -DNA/RNA <sup>™</sup> MagBead	<b>R2130</b> (96 prep)	<b>R2131</b> (4 x 96 prep)
DNA/RNA Shield <sup>™</sup> (2X concentrate)	25 ml	125 ml
Proteinase K <sup>1</sup> (lyophilized) & Storage Buffer	20 mg	20 mg (x4)
DNA/RNA Lysis Buffer	50 ml (x2)	200 ml (x2)
MagBinding Beads	6 ml	24 ml
MagBead DNA/RNA Wash 1 <sup>2</sup> (concentrate)	30 ml (x3)	120 ml (x3)
MagBead DNA/RNA Wash 2 <sup>3</sup> (concentrate)	20 ml (x3)	80 ml (x3)
DNase I <sup>1</sup> (lyophilized)	250 U (x3)	1500 U (x2)
DNA Digestion Buffer	4 ml	4 ml
DNA/RNA Prep Buffer	50 ml (x2)	200 ml (x2)
DNase/RNase-Free Water	30 ml	100 ml
Instruction Manual	1 pc	1 pc

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

<sup>1</sup> Reconstitute the lyophilized Proteinase K and DNase I according to Buffer Preparation, page 5.

<sup>2</sup> Add 20 ml (R2130) or 80 ml (R2131) of isopropanol to the MagBead DNA/RNA Wash 1 (concentrate).

<sup>3</sup> Add 30 ml (R2130) or 120 ml (R2131) of isopropanol to the MagBead DNA/RNA Wash 2 (concentrate).

### **Specifications**

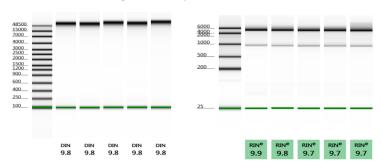
- Sample Sources Any cells (animal, bacterial, blood cells, etc.), all tissues (tough-to-lyse, FFPE, etc.), blood, biological fluids, environmental (plant/seed), swabs (stool, soil, microbial samples), and samples in DNA/RNA Shield™ or other preservation reagents.
- Sample Preservation and Inactivation DNA/RNA Shield<sup>™</sup>
  lyses cells, inactivates nucleases and infectious agents (e.g.,
  virus, pathogens) and is ideal for safe sample storage and
  transport at ambient temperatures (page 13).
- Size Genomic DNA (≥ 40 kb), mitochondrial and viral DNA (if present) and total RNA including small/microRNAs (≥ 17 nt).
- Purity A<sub>260</sub>/A<sub>280</sub> & A<sub>260</sub>/A<sub>230</sub> > 1.8. DNA & RNA are ready for Next-Gen Sequencing, RT/qPCR, etc.
- Binding Capacity 15 μg DNA/RNA per 30 μl MagBinding Beads.
- Compatibility For samples stored in preservation reagents: DNA/RNA Shield<sup>™</sup>, RNAprotect<sup>®</sup>, Allprotect<sup>®</sup>, Universal transport medium/viral transport medium (UTM<sup>®</sup>/VTM<sup>®</sup>), PAXgene<sup>®</sup> and RNAlater<sup>™</sup>.
- Elution Volume ≥ 50 µl DNase/RNase-Free Water.
- Equipment Needed (user provided) Magnetic stand or separator, heat block, liquid handler, or robotic sample processer (user provided) and nuclease-free tubes.
- Recommended Materials (sold 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), DNase/RNase-Free Tubes (1.5 ml; C2001).

### **Product Description**

The **Quick-DNA/RNA**™ **MagBead** kit provides a high-throughput, magnetic bead-based purification of both high-quality DNA and total RNA (including small/microRNAs) from the same starting sample. The provided **DNA/RNA Shield**™ inactivates infectious agents and is ideal for sample storage at ambient temperatures.

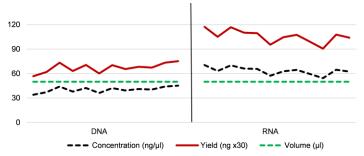
The extraction method has the option to recover total nucleic acids in one elution or DNA & RNA in separate elutions without the use of phenol. DNA/RNA is eluted into  $\geq$  50  $\mu$ l of **DNase/RNase-Free Water** and is ready for any downstream application including Next-Gen Sequencing, RT/PCR, hybridization, *etc.* 

**High-Quality DNA & RNA** 



DNA (left) and RNA (right) quality assessed using Agilent 2200 TapeStation. DNA and RNA were purified from HeLa cells using the *Quick-DNA/RNA* MagBead.

#### Reproducible Sample Processing



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

### Input Capacity and gDNA/Total RNA Yield

Input	Average gDNA Yield	Average RNA Yield	Kit Capacity
Cells	0.4 μg (per 10 <sup>5</sup> cells)	1 μg (per 10 <sup>5</sup> cells)	Up to 10 <sup>6</sup>
HeLa	0.6 µg	1.5 µg	
High Yield Tissue <sup>1 (mouse)</sup>	≥ 3 µg (per 1 mg)	≥ 3 µg (per 1 mg)	Up to 2 mg
Spleen	5-7 μg	3-5 µg	
Liver	1.5-3 µg	4-6 μg	
Low Yield Tissue <sup>1 (mouse)</sup>	≤ 3 µg (per 1 mg)	≤ 3 µg (per 1 mg)	Up to 5 mg
Brain, Heart	0.5-1.5 μg	0.5-1.5 μg	
Muscle	0.5-1.5 μg	0.5 <b>-</b> 2 μg	
Lung	1.5-3 µg	1-2 µg	
Intestine	1.5-3 µg	1-3 µg	
Kidney	1.5-3 µg	2-3 µg	
Whole Blood <sup>2</sup>	(per 100 μl)	(per 100 µl)	Up to 200 µl
Porcine	0.5-1 μg	1-2 µg	
Human	0.2 <b>-</b> 0.5 μg	0.2-1 μg	

<sup>1</sup> Yield from tissue can vary due to other factors (i.e., organism type, physiological state, and growth conditions. 2 Yield from blood can vary based upon collection, sample preparation, donor, age, and/or health conditions.

#### **Protocol**

The protocol consists of: (I) Buffer Preparation, (II) Sample Preparation, (III) Total Nucleic Acid Purification and/or (IV) DNA and RNA Purification

#### (I) Buffer Preparation

- Add 20 ml (R2130) or 80 ml (R2131) isopropanol to the MagBead DNA/RNA Wash 1 concentrate.
- ✓ Add 30 ml (R2130) or 120 ml (R2131) isopropanol to the MagBead DNA/RNA Wash 2 concentrate.
- ✓ To prepare 1X solution of DNA/RNA Shield<sup>™</sup>, mix equal amounts of the supplied 2X concentrate with nuclease-free water (not provided) and mix well.
- Reconstitute lyophilized Proteinase K at 20 mg/ml with Proteinase K Storage Buffer and mix by vortexing. Use immediately or store frozen aliquots:
  - **#D3001-2-20 (20 mg)**, add 1.04 ml buffer
- Reconstitute <u>each</u> vial of lyophilized **DNase I** with **DNase/RNase-Free Water** in a conical tube (not provided). Mix by gentle inversion and store frozen aliquots.

**#E1011-A (1500 U)**, add 13.5 ml water **#E1009-A (250 U)**, add 2.25 ml water

For <u>each</u> sample to be treated, prepare **DNase I Reaction Mix** (scale up proportionally): Add 45  $\mu$ I **DNase I** (reconstituted) and 5  $\mu$ I **DNA Digestion Buffer** in a nuclease-free tube (not provided), mix by gentle inversion and place on ice until ready to use.

#### (II) Sample Preparation

✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

# Samples stabilized and stored in DNA/RNA Shield<sup>™</sup> (cells, tissue, swab, etc.)

If frozen, thaw homogenized sample in **DNA/RNA Shield**<sup> $^{\text{M}}$ </sup> to room temperature (20-30°C). Mix well by vortex. Proceed to the appropriate procedure below based on sample type (omit the step involving the addition of DNA/RNA Shield $^{^{\text{M}}}$ ).

#### Cells & Tissue (mammalian)

- For samples (cells or tissue) already stored in DNA/RNA Shield<sup>™</sup>, add an equal volume of DNA/RNA Lysis Buffer (1:1), mix well and proceed to Total Nucleic Acid Purification, page 10 or DNA & RNA Purification, page 11.
- Cells: If in suspension¹, pellet by centrifugation (≤ 500 x g for 1 minute), remove supernatant and resuspend cell pellet in DNA/RNA Shield™ (1X)² (see table below). Proceed to Total Nucleic Acid Purification, page 10 or DNA & RNA Purification, page 11.

Cells	Add DNA/RNA Shield (1X)	
≤ 10 <sup>6</sup>	≥ 200 µl	

3. <u>Tissue</u><sup>3</sup>: Submerge an appropriate amount of fresh or frozen sample (see table below) into **DNA/RNA Shield**<sup>™</sup> (1X)<sup>2</sup> and homogenize<sup>4,5</sup>.

Tissue	Add DNA/RNA Shield (1X)
High-yield (≤ 2 mg) Low-yield (≤ 5 mg)	≤ 600 µl

- a. For every 200 µl of sample, add 10 µl **Proteinase K**. Mix and incubate at room temperature (20-30°C) for ≥ 30 minutes (homogenized) or 2-5 hours (non-homogenized). Optimization may be required.
- b. To remove particulate debris, centrifuge and transfer every 200 µl of the supernatant into a nuclease-free tube (not provided).
- Proceed to Total Nucleic Acid Purification, page 10 or DNA & RNA Purification, page 11.

<sup>1</sup> If liquid/media cannot be removed, add ≥ 3 volumes DNA/RNA Lysis Buffer to 1 volume liquid sample (3:1) and mix well. Proceed to Total Nucleic Acid Purification, page 10 or DNA & RNA Purification, page 11.

<sup>2</sup> For a 1X solution of **DNA/RNA Shield**<sup>™</sup>, see Buffer Preparation, page 5.

<sup>3</sup> For examples of sample type input and average yield, see chart on page 4.

<sup>4</sup> For efficient homogenization, bead beat samples with ZR BashingBead Lysis Tubes (S6012, S6003), sold separately. See Appendices (page 14) for bead beating parameters. Other types of homogenization can include mortar/pestle, dounce, syringe or tissue grinder, etc.

<sup>5</sup> Alternatively (if no homogenization), tissue samples can be Proteinase K treated only (proceed to step 3a).

# <u>Tough-to-Lyse Samples</u> (bacteria, yeast, insect, swab, soil<sup>1</sup>, stool<sup>1</sup>, plant<sup>1</sup>, seed<sup>1</sup>)

 Add 800 µl of **DNA/RNA Shield™** (1X)<sup>2</sup> to an appropriate amount of sample (see table below) and homogenize³ (e.g., bead beating).

Solid Tissue	Microbes	Add DNA/RNA Shield (1X)
Plant/Seed or Insect (≤ 20 mg)	Bacteria (≤ 2x10 <sup>8</sup> ) Yeast (≤ 2x10 <sup>7</sup> ) Swab, Stool/Soil (≤ 10 mg)	800 µl

- After homogenization, remove particulate debris by centrifugation at max speed. Transfer every 200 µl of the cleared supernatant into a nuclease-free tube (not provided).
- 3. Proceed to Total Nucleic Acid Purification, page 10 or DNA & RNA Purification, page 11.

#### FFPE Tissue

- 1. Remove (trim) excess paraffin wax from ≤ 5 mg FFPE tissue and transfer into a nuclease-free tube (not provided).
- Add 400 μl Deparaffinization Solution<sup>4</sup> to the sample. Incubate at 55°C for 1 minute. Vortex briefly. Remove the Deparaffinization Solution.
- 3. Add 95 μl **DNase/RNase-Free Water**, 95 μl **2X Digestion Buffer**<sup>4</sup>, and 10 μl **Proteinase K**. Mix well.
- 4. Incubate at 55°C for 1 hour. Then incubate at 65°C for 15 minutes to de-crosslink the sample.
- 5. Centrifuge to remove insoluble debris and transfer every 200 μl supernatant to a nuclease-free tube (not provided).
- 6. Proceed to Total Nucleic Acid Purification, page 10 or DNA & RNA Purification, page 11.

#### Blood Cells (mammalian, PBMCs, WBCs, etc.)

For blood cells, buffy coat and pelleted PAXgene<sup>®</sup> or RNAlater<sup>™</sup> samples, resuspend in **DNA/RNA Shield** (1X)<sup>2</sup>.

Blood Cells	Add DNA/RNA Shield™ (1X)
≤ 0.5 ml blood (≤ 10 <sup>6</sup> cells)	200 μΙ

2. For every 200 μl of sample, add 10 μl **Proteinase K**. Continue to step 3, page 8.

<sup>1</sup> For PCR inhibitor removal, use OneStep PCR Inhibitor Removal Kit (D6030).

<sup>2</sup> For a 1X solution of **DNA/RNA Shield™**, see Buffer Preparation, page 5.

<sup>3</sup> For efficient homogenization, bead beat samples with ZR BashingBead Lysis Tubes (S6012, S6003), sold separately. See Appendices (page 14) for bead beating parameters.

<sup>4</sup> Deparaffinization Solution (D3067-1-20) and 2X Digestion Buffer (D3050-1-20) are sold separately.

- 3. Mix and incubate at room temperature (20-30°C) for ≥ 30 minutes. Optimization may be required.
- 4. After incubation, vortex sample and centrifuge at max speed for 2 minutes to pellet debris. Transfer every 200 µl of the cleared supernatant to a nuclease-free tube (not provided).
- 5. Proceed to Total Nucleic Acid Purification, page 10 or DNA & RNA Purification, page 11.

#### Whole Blood<sup>1</sup> (mammalian)

- Add 200 µl DNA/RNA Shield™ (2X concentrate) directly to each 200 µl of fresh or frozen blood sample and mix thoroughly.
- 2. For every 400 μl of reagent/blood mixture, add 8 μl **Proteinase K** and mix well. Incubate at room temperature (20-30°C) for 30 minutes.
- After incubation, vortex sample and centrifuge at max speed for 2 minutes to pellet debris. Transfer the cleared supernatant to a new nuclease-free tube (not provided).
- 4. Add an equal volume of isopropanol (1:1) and mix well.
- Transfer 800 µl of the sample mixture into a new plate/tube and proceed to Total Nucleic Acid Purification, page 10, step 3. DNA & RNA Purification is not compatible.

#### Saliva & Buccal Cells

 For saliva and buccal cell samples, add an equal volume of DNA/RNA Shield™ (2X) (1:1).

Saliva & Buccal Cells	Add DNA/RNA Shield <sup>™</sup> (2X)
100 µl (≤ 5 x 10 <sup>6</sup> cells)	100 μΙ

- 2. For every 200 μl of reagent/sample mixture, add 10 μl **Proteinase K** and 20 μl **PK Digestion Buffer**.
- Mix and incubate at room temperature (20-30°C) for ≥ 30 minutes.
   Optimization may be required.
- 4. After incubation, vortex sample and centrifuge at max speed for 2 minutes to pellet debris. Transfer 200 μl of the cleared supernatant to a nuclease-free tube (not provided).
- 5. Proceed to Total Nucleic Acid Purification, page 10 or DNA & RNA Purification, page 11.

<sup>1</sup> Compatible with commonly used anticoagulants (e.g., EDTA, citrate, heparin)

#### **Urine**<sup>1</sup>

Generate pellet from up to 20 ml urine by adding 35 µl Urine
 Conditioning Buffer² for every 1 ml of urine and mix by vortex.
 Centrifuge at 3,000 x g for 15 minutes. Discard the supernatant and keep the pellet. Add DNA/RNA Shield™ (1X)³ and mix by pipetting.

Pelleted cells from urine	Add DNA/RNA Shield™ (1X)
≤ 20 ml urine	200 μΙ

- For every 200 μl of sample, add 10 μl Proteinase K.
- 3. Mix and incubate at room temperature (20-30°C) for ≥ 30 minutes. Optimization may be required.
- After incubation, vortex sample and centrifuge at max speed for 2 minutes to pellet debris. Transfer 200 μl of the cleared supernatant to a nuclease-free tube (not provided).
- Proceed to Total Nucleic Acid Purification, page 10 or DNA & RNA Purification, page 11.

<sup>1</sup> Warm up urine sample at 37°C for 5-10 minutes if there is visual precipitation or cloudiness. Samples that contain bacterial contamination will not be clear.

<sup>2</sup> Urine Conditioning Buffer (D3061-1-8, D30601-1-140) is sold separately.

<sup>3</sup> For a 1X solution of **DNA/RNA Shield**<sup>™</sup>, see Buffer Preparation, page 5.

#### (III) Total Nucleic Acid Purification

- Add 200 μl (1 volume) DNA/RNA Lysis Buffer to 200 μl sample and mix well<sup>1</sup>.
- 2. Add 400 µl ethanol (95-100%) to the sample and mix well<sup>1</sup>.
- 3. Add 30 µl **MagBinding Beads** and mix well<sup>1</sup> 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<sup>2</sup> until beads have pelleted, then aspirate<sup>3</sup> and discard the cleared supernatant.
- 5. Add 500 μl **MagBead DNA/RNA Wash 1** and mix well<sup>1</sup>. Pellet the beads<sup>2,3</sup> and discard the supernatant.
- 6. Add 500 μl **MagBead DNA/RNA Wash 2** and mix well<sup>1</sup>. Pellet the beads<sup>2,3</sup> and discard the supernatant.
- 7. Add 500 µl ethanol (95-100%) and mix well<sup>1</sup>. Pellet the beads<sup>2,3</sup> and discard the supernatant.
- Repeat step 7.
- 9. **DNase I** treatment (optional)
  - (D1) Add 50  $\mu$ I **DNase I Reaction Mix** and mix gently for 10 minutes.
  - (D2) Add 500 μl DNA/RNA Prep Buffer and mix well<sup>1</sup> for 10 minutes. Pellet the beads<sup>2,3</sup> and discard the supernatant.
  - (D3) Repeat steps 7-8.
- 10. Dry the beads for 10 minutes or until dry<sup>4</sup>.
- 11. To elute DNA/RNA from the beads, add ≥ 50 µl **DNase/RNase-Free Water** and mix well¹ for 5 minutes.
- 12. Transfer the plate/tube to the magnetic stand<sup>2</sup> until beads have pelleted, then aspirate<sup>3</sup> and dispense the eluted DNA/RNA to a new plate/tube.

The eluted DNA/RNA can be used immediately or stored frozen.

<sup>1</sup> 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.

<sup>2</sup> Use a strong-field magnetic stand or separator (e.g., ZŘ-96 MagStand, P1005; sold separately) until beads have pelleted.

<sup>3</sup> 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.

<sup>4</sup> 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).

#### (IV) DNA and RNA Purification (in two separate fractions)

- 1. Add 500  $\mu$ l (2.5 volumes) **DNA/RNA Lysis Buffer** to the 200  $\mu$ l sample and mix well<sup>1</sup>.
- 2. Add 30 μI **MagBinding Beads** and mix well for 20 minutes. Important: **MagBinding Beads** settle quickly, ensure that beads are kept in suspension while dispensing.
- Transfer the plate/tube to the magnetic stand<sup>2</sup> until beads (DNA)
  have pelleted, then transfer<sup>3</sup> the cleared supernatant (RNA) into a
  new plate/tube.

#### **DNA Purification** (beads)

- Add 500 μl MagBead
   DNA/RNA Wash 1 and mix well<sup>1</sup>. Pellet the beads<sup>2,3</sup> and discard the supernatant.
- 5. Add 500 µl MagBead

  DNA/RNA Wash 2 and mix

  well<sup>1</sup>. Pellet the beads<sup>2,3</sup> and
  discard the supernatant.
- Add 500 µl ethanol (95-100%) and mix well<sup>1</sup>. Pellet the beads<sup>2,3</sup> and discard the supernatant.
- 7. Repeat step 6.
- 8. Dry the beads for 10 minutes or until dry<sup>4</sup>.

#### RNA Purification (supernatant)

- Add 700 µl (1 volume) ethanol (95-100%) (1:1) to the supernatant and mix well¹.
- Add 30 μl/well MagBinding Beads and mix well<sup>1</sup> for 10 minutes.
- Transfer the plate/tube to the magnetic stand<sup>2</sup> until beads have pelleted, then aspirate<sup>3</sup> and discard the cleared supernatant.
- 7. Add 500 µl MagBead

  DNA/RNA Wash 1 and mix

  well<sup>1</sup>. Pellet the beads<sup>2,3</sup> and
  discard the supernatant.
- Add 500 μl MagBead DNA/RNA Wash 2 and mix well<sup>1</sup>. Pellet the beads<sup>2,3</sup> and discard the supernatant.

(Continue DNA and RNA Purification, page 12)

<sup>1</sup> 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.

<sup>2</sup> Use a strong-field magnetic stand or separator (e.g., ZR-96 MagStand, P1005; sold separately) until beads have pelleted.

<sup>3</sup> 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.

<sup>4</sup> 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).

#### DNA Purification (beads)

- Add 50 µl DNase/RNase-Free Water and mix well<sup>1</sup> for 5 minutes.
- Transfer the plate/tube to the magnetic stand<sup>2</sup> until beads have pelleted, then aspirate<sup>3</sup> and dispense the eluted DNA to a new plate/tube.

#### RNA Purification (supernatant)

- 9. Add 500 µl ethanol (95-100%) and mix well<sup>1</sup>. Pellet the beads<sup>2,3</sup> and discard the supernatant.
- 10. Repeat step 9.
- 11. **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<sup>1</sup> for 10
    minutes. Pellet the beads<sup>2,3</sup>
    and discard the supernatant.
  - (D3) Repeat steps 9-10.
- 12. Dry the beads for 10 minutes or until dry<sup>4</sup>
- 13. Add 50 μl **DNase/RNase- Free Water** and mix well<sup>1</sup> for 5 minutes.
- 14. Transfer the plate/tube to the magnetic stand<sup>2</sup> until beads have pelleted, then aspirate<sup>3</sup> and dispense the eluted RNA to a new plate/tube.

The eluted DNA and RNA can be used immediately or stored frozen.

### **Appendices**

#### Sample stabilization and storage in DNA/RNA Shield™

<u>Liquid samples (e.g., whole-blood)</u>: Add 3 volumes **DNA/RNA Shield**<sup>™</sup> (1X)<sup>1</sup> to 1 volume sample (3:1). Mix well.

Solid samples (e.g., tissue): Submerge sample (not to exceed 10% (v/v or w/v)) in **DNA/RNA Shield**™ (1X)¹ and homogenize (see Appendices, page 14).

Store samples in **DNA/RNA Shield**<sup> $^{\text{M}}$ </sup> at ambient temperature for  $\geq 1$  month or long term at frozen temperature. **DNA/RNA Shield**<sup> $^{\text{M}}$ </sup> is directly compatible with most guanidinium-based extraction methods (e.g., no need to remove reagent from the stored sample prior to extraction).

# <u>Samples in RNAprotect, Allprotect, RNAlater, PAXgene, UTM/VTM, saline or PBS</u>

- ✓ RNAProtect®, Allprotect®: Add 3 volumes **DNA/RNA Lysis Buffer** to 1 volume of liquid sample (3:1). Mix well and/or homogenize base on sample type (Sample Preparation, page 6). Proceed to Total Nucleic Acid Purification, page 10 or DNA & RNA Purification, page 11.
- ✓ RNAlater™:
  - a. Cells Pellet<sup>2</sup> by centrifugation at up to 5,000 x g and remove RNAlater (supernatant). Proceed to Sample Preparation, page 6.
  - b. Tissue Transfer into a new tube with forceps and remove any excess RNAlater<sup>™</sup>. Proceed to Sample Preparation, page 6.

Alternatively, for liquid samples from which RNAlater cannot be removed, add 1 volume of nuclease-free water (or PBS) to 1 volume sample (1:1) and mix. Then add 4 volumes **DNA/RNA Lysis Buffer** to 1 volume sample/water (or PBS) mixture (4:1). Mix well and go to Total Nucleic Acid Purification, page 10 or DNA & RNA Purification, page 11.

- ✓ <u>PAXgene®</u>: Refer to manufacturer's instructions to remove the reagent then proceed to Sample Preparation, Blood Cells, page 7.
- ✓ <u>Swab samples in UTM®/VTM®</u>, saline or PBS: Remove swab and add 3 volumes of **DNA/RNA Lysis Buffer** to 1 volume sample (3:1). Mix and aliquot every 200 µl of mixture into a nuclease-free tube. Proceed to Total Nucleic Acid Purification, page 10, step 2 or DNA and RNA Purification, page 11, step 2.

Optional: To inactivate pathogens, store at room temperature prior to purification, add 1 volume of DNA/RNA Shield™ (2X concentrate) to 1 volume liquid sample (1:1) and mix well. Then proceed to Sample Preparation, Samples in DNA/RNA Shield™, page 6.

<sup>1</sup> For a 1X solution of **DNA/RNA Shield**<sup>™</sup>, see Buffer Preparation, page 5.

<sup>2</sup> Different cells may react differently to centrifugation forces, and it is recommended to test the pelleting procedure with non-valuable samples first. Diluting RNAlater™ by 50% with cold PBS reduces solution density allowing for lower forces during cell pelleting (e.g., 500 x g).

(Appendices continued)

# <u>Liquids/Reaction Clean-up</u> (DNase I treated RNA, in vitro transcriptions, etc.)

Add 150  $\mu$ l **DNA/RNA Lysis Buffer** to a  $\geq$  50  $\mu$ l liquid sample (3:1) and mix well. Then add an equal volume of ethanol (95-100%) (e.g., 150  $\mu$ l ethanol to 150  $\mu$ l mixture) and mix well. Proceed to page 11, RNA Purification (supernatant), step 5.

#### Homogenization with ZR BashingBead Lysis Tubes

- Recommended for complete and efficient homogenization of tough-to-lyse samples (e.g., tissue, plant, seed, microbes, etc.). Lysis tubes sold separately.
- ✓ For high-speed (e.g., MP Bio FastPrep-24, Bertin Precellys) and low-speed homogenizers (e.g., Vortex Genie), bead-beating time optimization may be required.

	Tissue		Microbes
Input	Mammalian	Plant/Seed or Insect	Bacteria, Swab, Yeast, Stool/Soil
Cat. no.	S6003	S6003	S6012
(lysis bead size)	(2.0 mm)	(2.0 mm)	(0.5 mm and 0.1 mm)
High-speed	30-60 sec	3-5 min	30-60 sec
Low-speed	3-5 min	15-20 min	5-10 min

#### **Automation Scripts**

The *Quick-DNA/RNA™* MagBead (R2130/R2131) is compatible with automated platforms. For automation scripts and related technical support, email <u>automation@zymoresearch.com</u>. In the subject line, please include "Automation Scripts", instrument used and the product catalog number.

<sup>1</sup> Unit definition – one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A<sub>260</sub> units/ml of reaction mixture at 25°C.

# **Ordering Information**

Product Description	Catalog No.	Size
Quick-DNA/RNA <sup>™</sup> MagBead	R2130 R2131	96 preps. 4 x 96 preps.

Individual Kit Components	Catalog No.	Amount
DNA/RNA Lysis Buffer	D7001-1-50 D7001-1-200	50 ml 200 ml
DNA/RNA Prep Buffer	D7010-2-50 D7010-2-200	50 ml 200 ml
MagBinding Beads	D4100-2-6 D4100-2-24	6 ml 24 ml
DNase/RNase-Free Water	W1001-6 W1001-30	6 ml 30 ml
DNase I Set (lyophilized) (250 U supplied with DNA Digestion Buffer, 4 ml)	E1010	1 set
DNA/RNA Shield <sup>™</sup> (2X concentrate)	R1200-25 R1200-125	25 ml 125 ml
PK Digestion Buffer	R1200-1-5 R1200-1-20	5 ml 20 ml
Proteinase K (lyophilized) & Storage Buffer	D3001-2-5 D3001-2-20	5 mg 20 mg
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
Collection Plate	C2002	2 plates
Elution Plate	C2003	2 plates
96-Well Plate Cover Foil	C2007-4	4
ZR-96 MagStand	P1005	1

## **Complete Your Workflow**

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

ZR BashingBead Lysis Tubes	
2.0 mm beads #S6003	Plant/animal tissue
0.1 + 0.5 mm beads #S6012	Microbes
0.1 + 2.0 mm beads #S6014	Microbes in tissue/insects

✓ For isolation of DNA/RNA from any sample:

Quick-DNA/RNA Plus kits	
Microprep Plus #D7005	From 1 cell and up
MagBeads #R2130	Automatable (Tecan, Hamilton, Kingfisher, etc.)

✓ For clean-up (purification) and concentration of any RNA sample. (e.g., from the aqueous phase of TRIzol® extractions) or from any enzymatic reaction (e.g., DNase I treated RNA):

RNA Clean & Concentrator kits	
Microprep #R1013-R1014	DNase I Set included
MagBeads #R1082	Automatable (Tecan, Hamilton, Kingfisher, etc.)

✓ For NGS:

Zymo-Seq RiboFree Total RNA Library Prep kit		
#R3000	12 preps	
#R3003	96 preps	

## **Troubleshooting Guide**

Problem	Possible Causes and Suggested Solutions		
Precipitation, viscous lysate	Incomplete lysis and/or high-mass input:		
iysate	- If precipitation occurs (upon adding ethanol to the lysate) or if the lysate is extremely viscous, increase the volume of DNA/RNA Shield™ and/or DNA/RNA Lysis Buffer to ensure complete lysis and homogenization until lysate is transparent (see image).		
Low purity (A <sub>260</sub> /A <sub>230</sub> nm, A <sub>260</sub> /A <sub>280</sub> nm)	Incomplete lysis and/or cellular debris:		
(1260/1230 1111, 1260/1280 1111)	<ul> <li>Increase the volume of DNA/RNA Shield and/or DNA/RNA Lysis Buffer for complete lysis and homogenization. Be sure to centrifuge any cellular debris and then process the cleared lysate.</li> </ul>		
	Washing of beads:		
	<ul> <li>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.</li> </ul>		
Low yield	Sample input:		
	<ul> <li>Too much input or incomplete lysis/homogenization can cause overloading and result in compromised nucleic acid recovery. Use less input material and/or increase the volume DNA/RNA Shield™ and/or DNA/RNA Lysis Buffer.</li> </ul>		
	High-protein content (blood, plasma/serum, etc.)		
	- Perform Proteinase K treatment to the sample prior to purification. See appropriate sample preparation protocol.		
	Increase binding time:		
	<ul> <li>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.</li> </ul>		
DNA contamination	To remove DNA:		
	- Perform DNase I treatment during purification (or post-purification, then re-purify the treated sample).		
	<ul> <li>For future preps, increase the volume of DNA/RNA Shield™ and/or DNA/RNA Lysis Buffer to ensure complete lysis and homogenization of the sample.</li> </ul>		
RNA degradation	To prevent RNA degradation:		
	-Immediately collect and lyse fresh sample into DNA/RNA Shield™ and/or DNA/RNA Lysis Buffer ensure stability. Homogenized samples can be stored frozen for later processing.		

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

## **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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