MagMAX™ *mir*Vana™ Total RNA Isolation Kit

USER GUIDE

Manual extraction of total RNA including small RNA

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ThermoFisher SCIENTIFIC

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Revision	Date	Description
C.0	December 2018	Update shaking speeds.
B.0	December 2015	Removed extra steps in the RNA binding instructions
A.0	May 2015	New document

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Product information

Product description

The MagMAX™ *mir*Vana™ Total RNA Isolation Kit is designed for isolation of total RNA, including microRNA, from a wide variety of sample matrices. The kit uses MagMAX™ magnetic-bead technology, ensuring reproducible recovery of high-quality RNA that is suitable for a broad range of applications, including TaqMan® miRNA Detection Assays.

These protocols describe manual isolation of RNA from tissues, cells, serum and plasma, whole blood, and urine.

Kit contents and storage

Table 1 MagMAX™ *mir*Vana™ Total RNA Isolation Kit (Cat. no. A27828, 96 reactions)

Contents	Amount	Storage
Box 1 of 2		
Proteinase K, 50 mg/mL	0.48 mL	–25°C to –15°C
Lysis/Binding Enhancer	0.96 mL	
TURBO DNase™, 20 U/µL	0.2 mL	
Box 2 of 2		
Lysis Buffer	115 mL	15°C to 30°C
PK Digestion Buffer	4.4 mL	
RNA Binding Beads[1]	2 mL	
Wash Solution 1 Concentrate[2]	20 mL	
Wash Solution 2 Concentrate[2]	60 mL	
Rebinding Buffer	4.8 mL	
MagMAX™ TURBO DNase™ Buffer	4.6 mL	
Elution Buffer	9.6 mL	
Processing Plate	1	

Contents	Amount	Storage
Elution Plates	2	15°C to 30°C
Plate Covers	4	

[1] Do not freeze the RNA Binding Beads.
[2] Final volume; see "Before first use: prepare Wash Solutions" on page 8.

Materials required but not supplied

Unless otherwise indicated, all materials are available from Life Technologies (**thermofisher.com**). MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Equipment	
Thermo Scientific™ Compact Digital Microplate Shaker	Fisher Scientific 11-676-337
Fisher Scientific™ Analog Vortex Mixer	Fisher Scientific 02-215-365
For tissue samples only PRO250 Homogenizer with 7 × 95 mm Saw Tooth Bottom Generator Probe	PRO Scientific 01-01250 and 02-07095
For tissue samples and cells only Heating block, 37°C	MLS
For serum, plasma, urine, and whole blood samples only One of the following incubators, or an equivalent incubator with slatted shelves and thermometer and able to reach 65°C:	
Economy Lab Incubator	Fisher Scientific S50441A
VWR™ Digital Mini Incubator	VWR 10055-006
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Magnetic Stand-96	Cat. no. AM10027
Consumables	
Nonstick, RNase-free Microfuge Tubes (1.5 mL)	Cat. no. AM12450
Nonstick, RNase-free Microfuge Tubes (2.0 mL)	Cat. no. AM12475
5–mL Culture tubes	MLS

Item	Source
Conical tubes (15 mL)	Cat. no. AM12500
Aerosol-resistant pipette tips	MLS
Reagent reservoirs	MLS
Reagents	
Isopropanol, 100% (molecular grade or higher)	MLS
Ethanol, 200 proof (absolute)	MLS
2-Mercaptoethanol	MLS
(Optional) Chloroform	MLS

Methods

Important procedural guidelines

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- When mixing samples by pipetting up and down, avoid creating bubbles.
- Cover the plate during the incubation and shaking steps to prevent spill-over and cross-contamination. The same Plate Cover can be used throughout the procedure, unless it becomes contaminated.
- If you use a titer plate shaker other than the Thermo Scientific™ Compact Digital Microplate Shaker, verify that:
  - The plate fits securely on your titer plate shaker.
  - The recommended speeds are compatible with your titer plate shaker. Ideal speeds should allow for thorough mixing without splashing.
- Volumes for reagent mixes are given per well. We recommend that you prepare master mixes for larger sample numbers. To calculate volumes for master mixes, refer to the per-well volume and add 5% overage.
- Lysed samples can be stored in Lysis Binding Mix at –20°C for up to 4 days before adding the Binding Beads Mix. Thaw frozen samples to room temperatures before use.

Before first use: prepare Wash Solutions

Prepare the Wash Solutions from the concentrates:

- Add 10 mL of isopropanol to Wash Solution 1 Concentrate, mix, and store at room temperature.
- Add 48 mL of ethanol to Wash Solution 2 Concentrate, mix, and store at room temperature.

Isolate RNA from tissue samples

Sample collection and storage

- Process tissue immediately, store them in RNA*later*™ Stabilization Solution, or freeze them in liquid nitrogen and store at –80°C.
- For ease of processing, we recommend pre-weighing and storing the tissue in pieces of incremental sizes, according to the following guidelines:
  - Increments of ≤50 mg for tissues containing low or normal level of cellular RNase.
  - Increments of ≤30 mg for tissues containing high level of cellular RNase, such as spleen or pancreas.

Before each use: prepare TURBO DNase™ Solution and Binding Beads Mix

- Pre-heat Elution Buffer to 37°C.
- Prepare the TURBO DNase™ Solution as indicated in the following table, mix, and store on ice until use.

Component	Volume per well
MagMAX™ TURBO DNase™ Buffer	48 µL
TURBO DNase™	2 µL
Total TURBO DNase™ Solution	50 µL

- Prepare the Binding Beads Mix as indicated in the following table, mix, and store on ice until use.

Component	Volume per well
RNA Binding Beads	10 µL
Lysis/Binding Enhancer	10 µL
Total Binding Beads Mix	20 µL

Lyse the tissue samples

1. Determine the size of tissue (in mg) to be homogenized.
2. Determine the amount of Lysis Binding Mix that is needed to homogenize the tissue, according to the following ratios:
   - For tissues containing low to normal levels of cellular RNase (for example, brain, heart, or liver), use 20 µL of Lysis Binding Mix for 1 mg of tissue (1:20 ratio).
     For example, use 200 µL of Lysis Binding Mix for 10 mg of tissue.
   - For tissues containing high levels of cellular RNase (for example, spleen or pancreas), use 40 µL of Lysis Binding Mix for 1 mg of tissue (1:40 ratio).
     For example, use 400 µL of Lysis Binding Mix for 10 mg of tissue.

   Note: Most mechanical homogenizers require a minimum volume of 200 µL. Therefore, we recommend processing no less than 10 mg when homogenizing.
3. Prepare sufficient Lysis Binding Mix, according to the following table.

Component	Volume for 100 µL of Lysis Binding Mix
Lysis Buffer	100 µL
2-Mercaptoethanol	0.7 µL
Total Lysis Binding Mix	~100 µL

4. Add tissue to the prepared Lysis Binding Mix.

5. Homogenize the tissue sample using standard homogenization procedures.

IMPORTANT! Make sure that the tissue homogenization is complete to ensure maximal RNA recovery. For the tissue input amounts listed previously, we recommend homogenizing for 30 seconds to fully break up the sample and ensure maximal RNA recovery and quality.

Bind the RNA to the RNA Binding Beads

If samples were frozen at the previous step, thaw them completely to room temperature before proceeding.

1. Vortex the lysates and transfer 100 µL to a separate well in the Processing Plate.

Table 2 Recommended tissue mass per 100 µL of Lysis Binding Mix

Samples	Levels of cellular RNase	
	Low to normal (for example, liver)	High (for example, spleen)
Frozen tissue	up to 10 mg	up to 2.5 mg
Tissue stored in RNA*later*™ Stabilization Solution	up to 5 mg	up to 2.5 mg

Note: Most mechanical homogenizers require a minimum of 200 µL. Therefore, we recommend processing no less than 10 mg of tissue when homogenizing.

2. *(Optional)* Add 10 µL of chloroform to each well.

IMPORTANT! The addition of chloroform is required for samples containing high level of RNase (spleen, pancreas).

3. Cover the plate and shake as indicated.

Time	Speed
5 minutes	1150 rpm (Speed 10) [1]

[1] Setting for Lab-Line™ shaker.

4. Add 100 µL of isopropanol to each sample, cover the plate, and shake as indicated.

Time	Speed
2 minutes	950 rpm (Speed 7) [1]

[1] Setting for Lab-Line™ shaker.

5. Add 20 µL of the prepared Binding Beads Mix to each sample and shake as indicated.

Time	Speed
5 minutes	950 rpm (Speed 7) [1]

[1] Setting for Lab-Line™ shaker.

Wash the RNA on the RNA Binding Beads

1. Place the Processing Plate on the Magnetic Stand-96 for 5 minutes or until the solution is clear.
2. Carefully aspirate and discard the supernatant without disturbing the RNA Binding Beads.
3. Remove the Processing Plate from the Magnetic Stand-96 and add 150 µL of Wash Solution 1 to each sample.
4. Cover and shake the Processing Plate as indicated.

Time	Speed
1 minute	950 rpm (Speed 7) [1]

[1] Setting for Lab-Line™ shaker.

5. Place the Processing Plate on the Magnetic Stand-96 for 1 minute or until the solution is clear.
6. Carefully aspirate and discard the supernatant without disturbing the RNA Binding Beads.
7. Repeat step 3–step 6 with 150 µL of Wash Solution 2.
8. Shake the uncovered Processing Plate as indicated to dry the RNA Binding Beads.

Time	Speed
2 minutes	1150 rpm (Speed 10) [1]

[1] Setting for Lab-Line™ shaker.

Note: Make sure not to overdry the beads, especially if you are not using the recommended magnetic stand.

Treat with TURBO DNase™ and rebind the RNA to the RNA Binding Beads

1. Add 50 µL of TURBO DNase™ Solution to each sample, cover the plate, and shake as indicated.

Time	Speed
15 minutes	1150 rpm (Speed 10) [1]

[1] Setting for Lab-Line™ shaker.

2. Add 50 µL of Rebinding Buffer and 100 µL of isopropanol to each sample.

IMPORTANT! Do not pre-mix the Rebinding Buffer and isopropanol. Add them separately to the samples.

3. Cover and shake the Processing Plate as indicated to rebind the RNA.

Time	Speed
5 minutes	950 rpm (Speed 7) [1]

[1] Setting for Lab-Line™ shaker.

4. Place the Processing Plate on the Magnetic Stand-96 for 5 minutes or until the solution is clear.
5. Carefully aspirate and discard the supernatant without disturbing the RNA Binding Beads.
6. Remove the Processing Plate from the Magnetic Stand-96 and add 150 µL of Wash Solution 2 to each sample.
7. Cover and shake the Processing Plate as indicated.

Time	Speed
1 minute	950 rpm (Speed 7) [1]

[1] Setting for Lab-Line™ shaker.

8. Place the Processing Plate on the Magnetic Stand-96 for 1 minute or until the solution is clear.
9. Carefully aspirate and discard the supernatant without disturbing the RNA Binding Beads.
10. Repeat step 6–step 9.

Elute the RNA

1. Shake the uncovered Processing Plate as indicated to dry the RNA Binding Beads.

Time	Speed
2 minutes	1150 rpm (Speed 10) [1]

[1] Setting for Lab-Line™ shaker.

Note: Make sure not to overdry the beads, especially if you are not using the recommended magnetic stand.

2. Add 20–100 µL of pre-heated Elution Buffer to each sample, cover the plate, and shake as indicated.

Time	Speed
2 minutes	1150 rpm (Speed 10) [1]

[1] Setting for Lab-Line™ shaker.

Note: Use the appropriate volume of Elution Buffer to achieve the desired final RNA concentration. The volume of Elution Buffer supplied in the kit is sufficient for 96 samples eluted with 100 µL each.

3. Place the Processing Plate on the Magnetic Stand-96 for 3 minutes or until the solution is clear.
4. Transfer the supernatant to the Elution Plate.

The purified samples are ready immediately for use. Alternatively, store the covered Elution Plate:

- On ice for up to 8 hours.
- At –20°C for long-term storage.

Isolate RNA from cells

Sample collection and storage

We recommend using up to 1×10^6 cells grown in 96-well or 24-well cell culture plates. Cells can be adherent or in suspension.

Cells can be collected by any method routinely used in the laboratory. We recommend the methods described in "Lyse the cells and bind the RNA to the RNA Binding Beads" on page 13 to prepare cells from adherent and liquid cultures.

Before each use: prepare TURBO DNase™ Solution and Binding Beads Mix

- Pre-heat Elution Buffer to 37°C.
- Prepare the TURBO DNase™ Solution as indicated in the following table, mix, and store on ice until use.

Component	Volume per well
MagMAX™ TURBO DNase™ Buffer	48 µL
TURBO DNase™	2 µL
Total TURBO DNase™ Solution	50 µL

- Prepare the Binding Beads Mix as indicated in the following table, mix, and store on ice until use.

Component	Volume per well
RNA Binding Beads	10 µL
Lysis/Binding Enhancer	10 µL
Total Binding Beads Mix	20 µL

Lyse the cells and bind the RNA to the RNA Binding Beads

1. Prepare sufficient Lysis Binding Mix, according to the following table.

Component	Volume per well
Lysis Buffer	99 µL
Isopropanol	100 µL
2-Mercaptoethanol	1 µL
Total Lysis Binding Mix	200 µL

2. Collect the cells according to the following methods:
   - **Adherent cells:** remove the media from the wells of a 96-well or 24-well culture plate containing up to 1 × 10^6 cells and add 200 µL of Lysis Binding Mix to each sample.
   - **Suspension cells:** pellet cells (up to 1 × 10^6) in a 96-well or 24-well culture plate by spinning the culture plate at 1000 × *g* for 4 minutes at 4°C, remove the media from the wells, and add 200 µL of Lysis Binding Mix to each sample.

   IMPORTANT! Add Lysis Binding Mix to the cells immediately after they have been harvested.

3. Lyse the samples by pipetting up and down 5 times.
4. Incubate for 5 minutes, then transfer the cell lysates from the cell culture plate to the Processing Plate.
5. Cover and shake the plate as indicated.

Time	Speed
3 minutes	950 rpm (Speed 7) [1]

[1] Setting for Lab-Line™ shaker.

6. Remove the plate from the shaker and add 20 µL of Binding Beads Mix to each sample.
7. Cover and shake as indicated.

Time	Speed
5 minutes	850 rpm (Speed 6) [1]

[1] Setting for Lab-Line™ shaker.

Wash the RNA on the RNA Binding Beads

1. Place the Processing Plate on the Magnetic Stand-96 for 5 minutes or until the solution is clear.
2. Carefully aspirate and discard the supernatant without disturbing the RNA Binding Beads.
3. Remove the Processing Plate from the Magnetic Stand-96 and add 150 µL of Wash Solution 1 to each sample.
4. Cover and shake the Processing Plate as indicated.

Time	Speed
1 minute	950 rpm (Speed 7) [1]

[1] Setting for Lab-Line™ shaker.

5. Place the Processing Plate on the Magnetic Stand-96 for 1 minute or until the solution is clear.
6. Carefully aspirate and discard the supernatant without disturbing the RNA Binding Beads.

7. Repeat step 3–step 6 with 150 µL of Wash Solution 2.

8. Shake the uncovered Processing Plate as indicated to dry the RNA Binding Beads.

Time	Speed
2 minutes	1150 rpm (Speed 10) [1]

[1] Setting for Lab-Line™ shaker.

Note: Make sure not to overdry the beads, especially if you are not using the recommended magnetic stand.

Treat with TURBO DNase™ and rebind the RNA to the RNA Binding Beads

1. Add 50 µL of TURBO DNase™ Solution to each sample, cover the plate, and shake as indicated.

Time	Speed
15 minutes	1050 rpm (Speed 9) [1]

[1] Setting for Lab-Line™ shaker.

2. Add 50 µL of Rebinding Buffer and 100 µL of isopropanol to each sample.

IMPORTANT! Do not pre-mix the Rebinding Buffer and isopropanol. Add them separately to the samples.

3. Cover and shake the Processing Plate as indicated to rebind the RNA.

Time	Speed
3 minutes	950 rpm (Speed 7) [1]

[1] Setting for Lab-Line™ shaker.

4. Place the Processing Plate on the Magnetic Stand-96 for 3 minutes or until the solution is clear.

5. Carefully aspirate and discard the supernatant without disturbing the RNA Binding Beads.

6. Remove the Processing Plate from the Magnetic Stand-96 and add 150 µL of Wash Solution 2 to each sample.

7. Cover and shake the Processing Plate as indicated.

Time	Speed
1 minute	950 rpm (Speed 7) [1]

[1] Setting for Lab-Line™ shaker.

8. Place the Processing Plate on the Magnetic Stand-96 for 1 minute or until the solution is clear.

9. Carefully aspirate and discard the supernatant without disturbing the RNA Binding Beads.

10. Repeat step 6–step 9.

Elute the RNA

1. Shake the uncovered Processing Plate for 2 minutes as indicated to dry the RNA Binding Beads.

Time	Speed
2 minutes	1050 rpm (Speed 9) [1]

[1] Setting for Lab-Line™ shaker.

Note: Make sure not to overdry the beads, especially if you are not using the recommended magnetic stand.

2. Add 50–100 µL of pre-heated Elution Buffer to each sample, cover the plate, and shake as indicated.

Time	Speed
3 minutes	1050 rpm (Speed 9) [1]

[1] Setting for Lab-Line™ shaker.

Note: Use the appropriate volume of Elution Buffer to achieve the desired final RNA concentration. The volume of Elution Buffer supplied in the kit is sufficient for 96 samples eluted with 100 µL each.

3. Place the Processing Plate on the Magnetic Stand-96 for 3 minutes or until the solution is clear.

4. Transfer the supernatant to the Elution Plate.

The purified samples are ready immediately for use. Alternatively, store the covered Elution Plate:

- On ice for up to 8 hours.
- At –20°C for long-term storage.

Isolate RNA from serum and plasma samples

Sample collection and storage

- Collect blood using proper venipuncture collection and handling procedures.
  - For plasma samples:
    - Use EDTA or sodium citrate anticoagulant tubes.
    - Prepare and collect plasma samples from whole blood using standard procedures.
  - For serum: use serum-specific collection tubes.

  Invert the tube to ensure thorough mixing.

Note: Heparin is not recommended as an anti-coagulant since it can cause inhibition of PCR reactions.

- *(Optional)* Store samples between –20°C and –80°C. We recommend storing serum and plasma samples in smaller volumes to prevent multiple freeze/thaw cycles.

Before each use: prepare TURBO DNase™ Solution

- Pre-heat a sufficient volume of Elution Buffer to 65°C.
- Prepare the TURBO DNase™ Solution as indicated in the following table, mix, and store on ice until use.

Component	Volume per well
MagMAX™ TURBO DNase™ Buffer	48 µL
TURBO DNase™	2 µL
Total TURBO DNase™ Solution	50 µL

Digest the samples with Proteinase K

1. Add 5 µL of Proteinase K to wells in a 96-well plate.
2. Add 100 µL of serum or plasma samples to each well containing Proteinase K.
3. Add 45 µL of PK Digestion Buffer to each sample.

   Note: Mix the PK Digestion Buffer gently before use. If the buffer appears cloudy, heat to 37°C for 5–10 minutes before use.
4. Cover and shake the plate as indicated.

Time	Speed
5 minutes	950 rpm (Speed 7) [1]

[1] Setting for Lab-Line™ shaker.

5. Incubate at 65°C for 30 minutes.

IMPORTANT! Arrange plates in the incubator to allow adequate flow around the plate wells, to ensure that the samples quickly reach and maintain the incubation temperature.

Lyse the samples and bind the RNA to the RNA Binding Beads

1. Prepare sufficient Lysis Binding Mix, according to the following table.

Component	Volume per well
Lysis Buffer	99 µL
2-Mercaptoethanol	1 µL
Total Lysis Binding Mix	100 µL

2. Add 100 µL of Lysis Binding Mix to each sample.
3. Add 20 µL of RNA Binding Beads to each sample.

4. Cover and shake the plate as indicated.

Time	Speed
7 minutes	700 rpm (Speed 4) [1]

[1] Setting for Lab-Line™ shaker.

5. Add 270 µL of isopropanol and mix by pipetting up and down 5 times.

6. Cover the plate and shake as indicated, then mix by pipetting up and down 5 times.

Time	Speed
15 minutes	400 rpm (Speed 2) [1]

[1] Setting for Lab-Line™ shaker.

Wash the RNA on the RNA Binding Beads

1. Place the Processing Plate on the Magnetic Stand-96 for 5 minutes or until the solution is clear.

2. Carefully aspirate and discard the supernatant without disturbing the RNA Binding Beads.

3. Remove the Processing Plate from the Magnetic Stand-96 and add 150 µL of Wash Solution 1 to each sample.

4. Cover and shake the Processing Plate as indicated.

Time	Speed
1 minute	950 rpm (Speed 7) [1]

[1] Setting for Lab-Line™ shaker.

5. Place the Processing Plate on the Magnetic Stand-96 for 1 minute or until the solution is clear.

6. Carefully aspirate and discard the supernatant without disturbing the RNA Binding Beads.

7. Repeat step 3–step 6 with 150 µL of Wash Solution 2.

8. Shake the uncovered Processing Plate as indicated to dry the RNA Binding Beads.

Time	Speed
5 minutes	1150 rpm (Speed 10) [1]

[1] Setting for Lab-Line™ shaker.

Note: Make sure not to overdry the beads, especially if you are not using the recommended magnetic stand.

Treat with TURBO DNase™ and rebind the RNA to the RNA Binding Beads

1. Add 50 µL of TURBO DNase™ Solution to each sample, cover the plate, and shake as indicated.

Time	Speed
15 minutes	1050 rpm (Speed 9) [1]

[1] Setting for Lab-Line™ shaker.

2. Add 50 µL of Rebinding Buffer and 100 µL of isopropanol to each sample.

IMPORTANT! Do not pre-mix the Rebinding Buffer and isopropanol. Add them separately to the samples.

3. Cover and shake the Processing Plate as indicated to rebind the RNA.

Time	Speed
5 minutes	950 rpm (Speed 7) [1]

[1] Setting for Lab-Line™ shaker.

4. Place the Processing Plate on the Magnetic Stand-96 for 5 minutes or until the solution is clear.
5. Carefully aspirate and discard the supernatant without disturbing the RNA Binding Beads.
6. Remove the Processing Plate from the Magnetic Stand-96 and add 150 µL of Wash Solution 2 to each sample.
7. Cover and shake the Processing Plate as indicated.

Time	Speed
30 seconds	950 rpm (Speed 7) [1]

[1] Setting for Lab-Line™ shaker.

8. Place the Processing Plate on the Magnetic Stand-96 for 1 minute or until the solution is clear.
9. Carefully aspirate and discard the supernatant without disturbing the RNA Binding Beads.
10. Repeat step 6–step 9.

Elute the RNA

1. Shake the uncovered Processing Plate as indicated to dry the RNA Binding Beads.

Time	Speed
5 minutes	1150 rpm (Speed 10) [1]

[1] Setting for Lab-Line™ shaker.

Note: Make sure not to overdry the beads, especially if you are not using the recommended magnetic stand.

2. Add 50 µL of pre-heated Elution Buffer to each sample, cover the plate, and shake as indicated.

Time	Speed
2 minutes	1050 rpm (Speed 9) [1]

[1] Setting for Lab-Line™ shaker.

3. Incubate the Processing Plate at 65°C for 5 minutes.

4. Shake the Processing Plate as indicated.

Time	Speed
2 minutes	1050 rpm (Speed 9) [1]

[1] Setting for Lab-Line™ shaker.

5. Place the Processing Plate on the Magnetic Stand-96 for 3 minutes or until the solution is clear.

6. Transfer the supernatant to the Elution Plate.

The purified samples are ready immediately for use. Alternatively, store the covered Elution Plate:

- On ice for up to 8 hours.
- At –20°C for long-term storage.

Isolate RNA from whole blood samples

Sample collection and storage

- Collect blood using proper venipuncture collection and handling procedures.
  - Use EDTA or sodium citrate anticoagulant tubes.
  - Invert the tube to ensure thorough mixing.

  Note: Heparin is not recommended as an anti-coagulant since it can cause inhibition of PCR reactions.
- *(Optional)* Store samples between –20°C and –80°C. We recommend storing samples in smaller volumes to prevent multiple freeze/thaw cycles.

Before each use: prepare TURBO DNase™ Solution and RNA Binding Beads

- Pre-heat a sufficient volume of Elution Buffer to 65°C.
- Prepare the TURBO DNase™ Solution as indicated in the following table, mix, and store on ice until use.

Component	Volume per well
MagMAX™ TURBO DNase™ Buffer	48 µL
TURBO DNase™	2 µL
Total TURBO DNase™ Solution	50 µL

- Prepare the Binding Beads Mix as indicated in the following table, mix, and store on ice until use.

Component	Volume per well
RNA Binding Beads	10 µL
Lysis/Binding Enhancer	10 µL
Total Binding Beads Mix	20 µL

Digest the samples with Proteinase K

1. Add 5 µL of Proteinase K to wells in a 96-well plate.
2. Add 50 µL of whole blood samples to each well containing Proteinase K.
3. Add 25 µL of PK Digestion Buffer to each sample.

   Note: Mix the PK Digestion Buffer gently before use. If the buffer appears cloudy, heat to 37°C for 5–10 minutes before use.

4. Cover and shake the plate as indicated.

Time	Speed
5 minutes	950 rpm (Speed 7) [1]

[1] Setting for Lab-Line™ shaker.

5. Incubate at 65°C for 10 minutes.

IMPORTANT! Arrange plates in the incubator to allow adequate flow around the plate wells, to ensure that samples quickly reach and maintain the incubation temperature.

Lyse the cells and bind the RNA to the RNA Binding Beads

1. Add 20 µL of Binding Beads Mix to each sample, cover the plate and shake as indicated.

Time	Speed
5 minutes	850 rpm (Speed 6) [1]

[1] Setting for Lab-Line™ shaker.

2. Prepare sufficient Lysis Binding Mix, according to the following table.

Component	Volume per well
Lysis Buffer	65 µL
2-Mercaptoethanol	0.65 µL
Total Lysis Binding Mix	~65 µL

3. Add 65 µL of Lysis Binding Mix to each sample.
4. Cover and shake the plate as indicated.

Time	Speed
5 minutes	800 rpm (Speed 5) [1]

[1] Setting for Lab-Line™ shaker.

5. Add 135 µL of isopropanol to each sample, cover the plate, and shake as indicated.

Time	Speed
10 minutes	700 rpm (Speed 4) [1]

[1] Setting for Lab-Line™ shaker.

Wash the RNA on the RNA Binding Beads

1. Place the Processing Plate on the Magnetic Stand-96 for 5 minutes.
2. Carefully aspirate and discard the supernatant without disturbing the RNA Binding Beads.
3. Remove the Processing Plate from the Magnetic Stand-96 and add 150 µL of Wash Solution 1 to each sample.
4. Cover and shake the Processing Plate as indicated.

Time	Speed
1 minute	950 rpm (Speed 7) [1]

[1] Setting for Lab-Line™ shaker.

5. Place the Processing Plate on the Magnetic Stand-96 for 1 minute or until the solution is clear.
6. Carefully aspirate and discard the supernatant without disturbing the RNA Binding Beads.
7. Repeat step 3–step 6 with 150 µL of Wash Solution 2.
8. Shake the uncovered Processing Plate as indicated to dry the RNA Binding Beads.

Time	Speed
2 minutes	1150 rpm (Speed 10) [1]

[1] Setting for Lab-Line™ shaker.

Note: Make sure not to overdry the beads, especially if you are not using the recommended magnetic stand.

Treat with TURBO DNase™ and rebind the RNA to the RNA Binding Beads

1. Add 50 µL of TURBO DNase™ Solution to each sample, cover the plate, and shake as indicated.

Time	Speed
15 minutes	1050 rpm (Speed 9) [1]

[1] Setting for Lab-Line™ shaker.

2. Add 50 µL of Rebinding Buffer and 100 µL of isopropanol to each sample.

IMPORTANT! Do not pre-mix the Rebinding Buffer and isopropanol. Add them separately to the samples.

3. Cover and shake the Processing Plate as indicated to rebind the RNA.

Time	Speed
5 minutes	950 rpm (Speed 7) [1]

[1] Setting for Lab-Line™ shaker.

4. Place the Processing Plate on the Magnetic Stand-96 for 5 minutes or until the solution is clear.

5. Carefully aspirate and discard the supernatant without disturbing the RNA Binding Beads.

6. Remove the Processing Plate from the Magnetic Stand-96 and add 150 µL of Wash Solution 2 to each sample.

7. Cover and shake the Processing Plate as indicated.

Time	Speed
30 seconds	950 rpm (Speed 7) [1]

[1] Setting for Lab-Line™ shaker.

8. Place the Processing Plate on the Magnetic Stand-96 for 1 minute or until the solution is clear.

9. Carefully aspirate and discard the supernatant without disturbing the RNA Binding Beads.

10. Repeat step 6–step 9.

Elute the RNA

1. Shake the uncovered Processing Plate as indicated to dry the RNA Binding Beads.

Time	Speed
5 minutes	1150 rpm (Speed 10) [1]

[1] Setting for Lab-Line™ shaker.

Note: Make sure not to overdry the beads, especially if you are not using the recommended magnetic stand.

2. Add 50 µL of pre-heated Elution Buffer to each sample, cover the plate, and shake as indicated.

Time	Speed
2 minutes	1150 rpm (Speed 10) [1]

[1] Setting for Lab-Line™ shaker.

3. Incubate the Processing Plate at 65°C for 5 minutes.

4. Shake the Processing Plate as indicated.

Time	Speed
2 minutes	1150 rpm (Speed 10) [1]

[1] Setting for Lab-Line™ shaker.

5. Place the Processing Plate on the Magnetic Stand-96 for 3 minutes or until the solution is clear.

6. Transfer the supernatant to the Elution Plate.

The purified samples are ready immediately for use. Alternatively, store the covered Elution Plate:

- On ice for up to 8 hours.
- At –20°C for long-term storage.

Isolate RNA from urine samples

Sample collection and storage

- Sample collection: Collect samples in a sterile container.
- *(Optional)* Sample storage:
  - Store at 4°C no longer than overnight.
  - Store at –80°C for long-term storage. We recommend storing samples in smaller volumes to prevent multiple freeze/thaw cycles.

Before each use: prepare TURBO DNase™ Solution and RNA Binding Beads

- Pre-heat a sufficient volume of Elution Buffer to 65°C.
- Prepare the TURBO DNase™ Solution as indicated in the following table, mix, and store on ice until use.

Component	Volume per well
MagMAX™ TURBO DNase™ Buffer	48 µL
TURBO DNase™	2 µL
Total TURBO DNase™ Solution	50 µL

- Prepare the Binding Beads Mix as indicated in the following table, mix, and store on ice until use.

Component	Volume per well
RNA Binding Beads	20 µL
Lysis/Binding Enhancer	10 µL
Total Binding Beads Mix	30 µL

Lyse the cells and bind the RNA to the RNA Binding Beads

1. Prepare sufficient Lysis Binding Mix, according to the following table.

Component	Volume per well
Lysis Buffer	198 µL
2-Mercaptoethanol	2 µL
Total Lysis Binding Mix	200 µL

2. Combine 250 µL of urine sample with 200 µL of Lysis Binding Mix in the Processing Plate.

3. Cover the plate and shake as indicated.

Time	Speed
7 minutes	500 rpm (Speed 3) [1]

[1] Setting for Lab-Line™ shaker.

4. Remove the plate from the shaker and add 30 µL of Binding Beads Mix to each sample.

5. Cover the plate and shake as indicated.

Time	Speed
5 minutes	500 rpm (Speed 3) [1]

[1] Setting for Lab-Line™ shaker.

6. Add 480 µL of isopropanol to each sample and pipet up and down 5 times, then shake as indicated.

Time	Speed
20 minutes	200 rpm (Speed 1) [1]

[1] Setting for Lab-Line™ shaker.

Wash the RNA on the RNA Binding Beads

1. Place the Processing Plate on the Magnetic Stand-96 for 5 minutes or until the solution is clear.

2. Carefully aspirate and discard the supernatant without disturbing the RNA Binding Beads.

3. Remove the Processing Plate from the Magnetic Stand-96 and add 150 µL of Wash Solution 1 to each sample.

4. Cover and shake the Processing Plate as indicated.

Time	Speed
1 minute	950 rpm (Speed 7) [1]

[1] Setting for Lab-Line™ shaker.

5. Place the Processing Plate on the Magnetic Stand-96 for 1 minute or until the solution is clear.

6. Carefully aspirate and discard the supernatant without disturbing the RNA Binding Beads.

7. Repeat step 3–step 6 with 150 µL of Wash Solution 2.

8. Shake the uncovered Processing Plate as indicated to dry the RNA Binding Beads.

Time	Speed
5 minutes	1150 rpm (Speed 10) [1]

[1] Setting for Lab-Line™ shaker.

Note: Make sure not to overdry the beads, especially if you are not using the recommended magnetic stand.

Treat with TURBO DNase™ and rebind the RNA to the RNA Binding Beads

1. Add 50 µL of TURBO DNase™ Solution to each sample, cover the plate, and shake as indicated.

Time	Speed
15 minutes	1050 rpm (Speed 9) [1]

[1] Setting for Lab-Line™ shaker.

2. Add 50 µL of Rebinding Buffer and 100 µL of isopropanol to each sample.

IMPORTANT! Do not pre-mix the Rebinding Buffer and isopropanol. Add them separately to the samples.

3. Cover and shake the Processing Plate as indicated to rebind the RNA.

Time	Speed
5 minutes	950 rpm (Speed 7) [1]

[1] Setting for Lab-Line™ shaker.

4. Place the Processing Plate on the Magnetic Stand-96 for 5 minutes or until the solution is clear.

5. Carefully aspirate and discard the supernatant without disturbing the RNA Binding Beads.

6. Remove the Processing Plate from the Magnetic Stand-96 and add 150 µL of Wash Solution 2 to each sample.

7. Cover and shake the Processing Plate as indicated.

Time	Speed
30 seconds	950 rpm (Speed 7) [1]

[1] Setting for Lab-Line™ shaker.

8. Place the Processing Plate on the Magnetic Stand-96 for 1 minute or until the solution is clear.

9. Carefully aspirate and discard the supernatant without disturbing the RNA Binding Beads.

10. Repeat step 6–step 9.

Elute the RNA

1. Shake the uncovered Processing Plate as indicated to dry the RNA Binding Beads.

Time	Speed
5 minutes	1150 rpm (Speed 10) [1]

[1] Setting for Lab-Line™ shaker.

Note: Make sure not to overdry the beads, especially if you are not using the recommended magnetic stand.

2. Add 50–60 µL of pre-heated Elution Buffer to each sample, cover the plate, and shake as indicated.

Time	Speed
2 minutes	1050 rpm (Speed 9) [1]

[1] Setting for Lab-Line™ shaker.

3. Incubate the Processing Plate at 65°C for 5 minutes, then shake as indicated.

Time	Speed
2 minutes	1050 rpm (Speed 9) [1]

[1] Setting for Lab-Line™ shaker.

4. Place the Processing Plate on the Magnetic Stand-96 for 3 minutes or until the solution is clear.

5. Transfer supernatant to the Elution Plate.

The purified samples are ready immediately for use. Alternatively, store the covered Elution Plate:

- On ice for up to 8 hours.
- At –20°C for long-term storage.

A Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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