MagMAX™ *mir*Vana™ Total RNA Isolation Kit

High-throughput isolation of RNA (including small RNA) from blood samples

Catalog Number A27828

Pub. No. MAN0011137 **Rev.** B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

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.

This protocol describes isolation of RNA from blood samples, optimized for use with the MagMAX™ Express-96 Deep Well Magnetic Particle Processor, the KingFisher™ Flex Magnetic Particle Processor 96DW (96-well deep well setting), or the KingFisher™ Duo Prime Magnetic Particle Processor (12-well deep well setting).

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	
Elution Plates	2	
Plate Covers	4	

[1] Do not freeze the RNA Binding Beads.

[2] Final volume; see "Before first use: prepare Wash Solutions" on page 2.

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
Magnetic particle processor, one of the following:	
MagMAX™ Express-96 Deep Well Magnetic Particle Processor	Cat. no. 4400079
KingFisher™ Flex Magnetic Particle Processor 96DW[1]	Thermo Scientific Cat. no. 5400630
KingFisher™ Duo Prime Magnetic Particle Processor[1]	Thermo Scientific Cat. no. 5400110
Other equipment	
Thermo Scientific™ Compact Digital Microplate Shaker	Fisher Scientific Cat. no. 11-676-337
Fisher Scientific™ Analog Vortex Mixer	Fisher Scientific Cat. no. 02-215-365
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 Cat. no. S50441A
VWR™ Digital Mini Incubator	VWR Cat. no. 10055-006
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Plates and combs[2]	
Deep Well Plates, one of the following:	
MagMAX™ Express-96 Deep Well Plates	Cat. no. 4388476
KingFisher™ Flex Microtiter Deepwell 96 Plate, Sterile	Thermo Scientific Cat. no. 95040460
Standard Well Plates, one of the following:	
MagMAX™ Express-96 Standard Plates	Cat. no. 4388475
KingFisher™ 96 KF Microplate	Thermo Scientific Cat. no. 97002540
One of the following tip combs, depending on the magnetic particle processor used:	
MagMAX™ Express-96 Deep Well Tip Combs	Cat. no. 4388487
KingFisher™ 96 Tip Comb for DW Magnets	Thermo Scientific Cat. no. 97002534
KingFisher™ Duo 12-Tip Comb, for Microtiter 96 Deepwell plate	Cat. no. 97003500
Other consumables	
MicroAmp™ Clear Adhesive Film	Cat. no. 4306311
Nonstick, RNase-free Microfuge Tubes (1.5 mL)	Cat. no. AM12450
Nonstick, RNase-free Microfuge Tubes (2.0 mL)	Cat. no. AM12475
Conical tubes (15 mL)	Cat. no. AM12500
Aerosol-resistant pipette tips	MLS
Reagent reservoirs	MLS

Item	Source
Reagents	
Isopropanol, 100% (molecular grade or higher)	MLS
Ethanol, 200 proof (absolute)	MLS
2-Mercaptoethanol	MLS

[1] See "If needed, download the KingFisher™ Flex or Duo program" on page 2

[2] KingFisher™ Duo Combi Pack (Cat. no. 97003530) includes plates and combs for the KingFisher™ Duo Prime Magnetic Particle Processor.

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.

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.

If needed, download the KingFisher™ Flex or Duo program

The program required for this protocol is not pre-installed on the KingFisher™ Flex Magnetic Particle Processor 96DW or on the KingFisher™ Duo Prime Magnetic Particle Processor.

1. On the MagMAX™ *mir*Vana™ Total RNA Isolation Kit web page, scroll down to the **Product Literature** section.
2. Right-click on the appropriate program for your instrument:
   - **A27828_FLEX_BioFluids** for KingFisher™ Flex Magnetic Particle Processor 96DW.
   - **A27828_DUO_BioFluids** for KingFisher™ Duo Prime Magnetic Particle Processor.
3. select **Save as Target** to download to your computer.
4. Refer to the manufacturer's documentation for instructions for installing the program on the instrument.

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.

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

- 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

Perform RNA extraction from blood samples

Isolate RNA using the MagMAX™ Express-96 Deep Well Magnetic Particle Processor or the KingFisher™ Flex Magnetic Particle Processor 96DW

1 **Digest the samples with Proteinase K**

a. Add 5 µL of Proteinase K to wells in a MagMAX™ Express or KingFisher™ Flex 96 Deep-Well Plate.
b. Add 50 µL of blood samples to each well containing Proteinase K.
c. 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.

d. Use a P200 multichannel pipette (set to 40 µL) to mix samples by gently pipetting up and down 10 times.
e. Cover and shake the plate as indicated.

Time	Speed
5 minutes	1050 rpm (Speed 8) [1]

[1] Setting for Lab-Line™ shaker.

f. 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.

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

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

Time	Speed
5 minutes	1050 rpm (Speed 8) [1]

[1] Setting for Lab-Line™ shaker.

b. 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

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

Time	Speed
5 minutes	1050 rpm (Speed 8) [1]

[1] Setting for Lab-Line™ shaker.

During the incubation, set up the processing plates (next section).

e. Add 135 µL of isopropanol.
f. Proceed directly to "Wash, rebind, and elute the RNA".

3 **Set up the processing plates**

While the samples are incubating, set up the Wash, DNase, Elution, and Tip Comb Plates outside the instrument as described in the following table.

Table 2 Processing plates

Plate ID	Plate position[1]	Plate type	Reagent	Volume per well
Wash Plate 1	2	Standard	Wash Solution 1	150 µL
Wash Plate 2	3	Standard	Wash Solution 2	150 µL
DNase Plate[2]	4	Deep Well	TURBO DNase™ Solution	50 µL
Wash Plate 3	5	Standard	Wash Solution 2	150 µL
Wash Plate 4	6	Standard	Wash Solution 2	150 µL
Elution Plate	7	Standard	Elution Buffer	50 µL
Tip Comb	8	Deep Well or standard	Place a MagMAX™ Express-96 Deep Well Tip Comb in a MagMAX™ Express-96 Deep Well Plate or in a MagMAX™ Express-96 Standard Plate.	

[1] Position on the instrument

[2] The instrument prompts the user to add 50 µL of Rebinding Buffer and 100 µL of isopropanol to the DNase Plate after the DNase treatment step.

4 **Wash, rebind, and elute the RNA**

a. Ensure that the instrument is set up for processing with the deep well magnetic head and select the program on the instrument.
   - **A27828_MME96_BioFluids** on MagMAX™ Express-96 Deep Well Magnetic Particle Processor
   - **A27828_FLEX_BioFluids** on KingFisher™ Flex Magnetic Particle Processor

b. Start the run and load the prepared processing plates in their positions when prompted by the instrument (see Table 2).

c. Load the sample plate (containing lysate, isopropanol, and Binding Beads Mix) at position 1 when prompted by the instrument.

d. When prompted by the instrument (30–35 minutes after the initial start):
   1. Remove the DNase Plate from the instrument.
   2. Add 50 µL of Rebinding Buffer and 100 µL of isopropanol to each sample well.
      Add Rebinding Buffer and isopropanol immediately after the prompt, to prevent excessive drying of any beads that are still captured on the Tip Comb.

      IMPORTANT! Do not pre-mix the Rebinding Buffer and isopropanol. Add them separately to the samples.
   3. Load the DNase Plate back onto the instrument, and press **Start**.

e. At the end of the run (approximately 60 minutes after the initial start), remove the Elution Plate from the instrument and seal immediately with a new MicroAmp™ Clear Adhesive Film.
   - *(Optional)* Eluates can be transferred to a storage plate after collection.
   - If excess bead residue is seen in the wells, place the Elution Plate on the Magnetic Stand-96 to capture any residue prior to downstream use of the RNA.

   IMPORTANT! Do not allow the purified samples to sit uncovered at room temperature for more than 10 minutes, to prevent evaporation and contamination.

The purified samples are ready for immediate use. Alternatively, store the covered Elution Plate:
- On ice for up to 8 hours.
- At –20°C or –80°C for long-term storage.

Isolate RNA using the KingFisher™ Duo Prime Magnetic Particle Processor

1 Digest the samples with Proteinase K

a. Add 5 µL of Proteinase K to wells in Row B of a MagMAX™ Express or KingFisher™ Flex 96 Deep-Well Plate.
b. Add 50 µL of blood samples to each well containing Proteinase K.
c. Add 25 µL of PK Digestion Buffer to each sample.
d. Use a P200 multichannel pipette (set to 40 µL) to mix samples by gently pipetting up and down 10 times.
e. Cover and shake the plate as indicated.

Time	Speed
5 minutes	1050 rpm (Speed 8) [1]

[1] Setting for Lab-Line™ shaker.

f. 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.

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

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

Time	Speed
5 minutes	1050 rpm (Speed 8) [1]

[1] Setting for Lab-Line™ shaker.

b. 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

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

Time	Speed
5 minutes	1050 rpm (Speed 8) [1]

[1] Setting for Lab-Line™ shaker.

e. Add 135 µL of isopropanol.

3 Set up the processing plate

Add processing reagents as indicated in the following table.

Table 3 Volume of processing reagents and plate location

Row ID	Plate row[1]	Reagent	Volume per well
Elution	A	Elution Buffer	50 µL
Wash 1	C	Wash Solution 1	150 µL
Wash 2	D	Wash Solution 2	150 µL
DNase[2]	E	TURBO DNase™ Solution	50 µL
Wash 3	F	Wash Solution 2	150 µL
Wash 4	G	Wash Solution 2	150 µL
Tip Comb	H	Place a KingFisher™ Duo 12-Tip Comb in Row H.	

[1] Row on the MagMAX™ Express-96 Deep Well Plate.
[2] The instrument prompts the user to add 50 µL of Rebinding Buffer and 100 µL of isopropanol to the DNase Plate after the DNase treatment step.

4 Wash, rebind, and elute the RNA

a. Ensure that the instrument is set up for processing with the deep well 96–well plates and select the program **A27828_DUO_BioFluids** on the instrument.
b. Start the run and load the prepared processing plate when prompted by the instrument (see Table 3).
c. When prompted by the instrument (approximately 30–35 minutes after initial start):
   1. Remove the plate from the instrument.
   2. Add 50 µL of Rebinding Buffer and 100 µL of isopropanol to each sample well in Row E.

      Add Rebinding Buffer and isopropanol immediately after the prompt, to prevent excessive drying of any beads that are still captured on the Tip Comb.

      IMPORTANT! Do not pre-mix the Rebinding Buffer and isopropanol. Add them separately to the samples.
   3. Load the plate back onto the instrument, and press **Start**.

4 Wash, rebind, and elute the RNA *(continued)*

d. At the end of the run (approximately 60 minutes after initial start), remove the Elution Plate from the instrument and transfer the eluted RNA (Row A) to an Elution Plate.

e. Seal immediately with a new MicroAmp™ Clear Adhesive Film.

IMPORTANT! Do not allow the purified samples to sit uncovered at room temperature for more than 10 minutes, to prevent evaporation and contamination.

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

- On ice for up to 8 hours.
- At –20°C or –80°C for long-term storage.

Limited product warranty

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