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. E.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), the KingFisher™ Apex with 96 Deep–Well head, and 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, 20 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 25°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	4.8 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 through thermofisher.com. MLS: Fisher Scientific (fisherscientific.com) or other major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Magnetic particle processor, one of the following:	
MagMAX™ Express-96 Deep Well Magnetic Particle Processor	—[1]
KingFisher™ Flex Magnetic Particle Processor 96DW[2]	5400630
KingFisher™ Apex with 96 Deep–Well head[2]	5400930
KingFisher™ Duo Prime Magnetic Particle Processor[2]	5400110
Other equipment	
Thermo Scientific™ Compact Digital Microplate Shaker	Fisher Scientific 11-676-337
Fisher Scientific™ Analog Vortex Mixer	Fisher Scientific 02-215-365
One of the following incubators, or an equivalent incubator with slatted shelves and thermometer and able to reach 65°C:	
Economy Standard Incubator, 19.8 L, aluminum	Fisher Scientific S50441A
Andwin Scientific Digital Mini Incubator	Fisher Scientific 50-112-6011
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Plates and combs[3]	
Deep-well plates, one of the following:	
KingFisher™ Flex Microtiter Deep-Well 96 plate, sterile	95040460
KingFisher™ 96 Deep-Well Plate, v-bottom, polypropylene	95040450
KingFisher™ 96 Deep-Well Plate, Barcoded	95040450B
Standard well plate:	
KingFisher™ 96 KF microplate	97002540
One of the following tip combs, depending on the magnetic particle processor used:	
KingFisher™ 96 tip comb for deep-well magnets	97002534
KingFisher™ 12-tip comb, for 96 deep-well plate[4]	97003500
Other consumables	
MicroAmp™ Clear Adhesive Film	4306311
Nonstick, RNase-free Microfuge Tubes, 1.5 mL	AM12450
Nonstick, RNase-Free Microfuge Tubes, 2.0 mL	AM12475
Conical Tubes (15 mL)	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] Not available for sale.

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

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

[4] For use with the KingFisher™ Duo Prime instrument only.

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 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™ Apex, Flex, or Duo program

The program required for this protocol is not pre-installed on the KingFisher™ instrument.

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.
   - **MagMAX_mirVana_Biofluids** for KingFisher™ Apex with 96 Deep–Well head.
   - **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

1. Add 5 µL of Proteinase K to wells in a KingFisher™ 96 Deep-Well Plate.
2. Add 50 µL of 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. Use a P200 multichannel pipette (set to 40 µL) to mix samples by gently pipetting up and down 10 times.
5. Cover and shake the plate as indicated.

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

[1] Setting for Lab-Line™ shaker.

1 Digest the samples with Proteinase K *(continued)*

6. 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

1. 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.

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	1050 rpm (Speed 8) [1]

[1] Setting for Lab-Line™ shaker.

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

5. Add 135 µL of isopropanol.
6. 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 KingFisher™ 96 tip comb for deep-well magnets in a KingFisher™ 96 Deep-Well Plate or in a KingFisher™ 96 KF microplate.	

[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

1. 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
2. Start the run and load the prepared processing plates in their positions when prompted by the instrument (see Table 2).
3. Load the sample plate (containing lysate, isopropanol, and Binding Beads Mix) at position 1 when prompted by the instrument.
4. When prompted by the instrument (30–35 minutes after the initial start):
   a. Remove the DNase Plate from the instrument.
   b. 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.

   c. Load the DNase Plate back onto the instrument, and press **Start**.
5. 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.

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

- 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™ Apex with 96 Deep–Well head

1 Digest the samples with Proteinase K

1. Add 5 µL of Proteinase K to wells in a KingFisher™ 96 Deep-Well Plate.
2. Add 50 µL of blood samples to each well containing Proteinase K.
3. Add 25 µL of PK Digestion Buffer to each sample.
4. Use a P200 multichannel pipette (set to 40 µL) to mix samples by gently pipetting up and down 10 times.

   Note: If the buffer appears cloudy, heat to 37°C for 5 to 10 minutes before use.
5. Cover and shake the plate as indicated.

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

[1] Setting for Lab-Line™ shaker.

6. Incubate at 65°C for 10 minutes.

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

2 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	1050 rpm (Speed 8) [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	1050 rpm (Speed 8) [1]

[1] Setting for Lab-Line™ shaker.

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

5. Add 135 µL of isopropanol.
6. 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 3 Processing plates

Plate ID	Plate position[1]	Plate type	Reagent	Volume per well
Wash Plate 1	3	Standard	Wash Solution 1	150 µL
Wash Plate 2	4	Standard	Wash Solution 2	150 µL
DNase Plate[2]	5	Deep Well	TURBO DNase™ Solution	50 µL
Wash Plate 3	6	Standard	Wash Solution 2	150 µL
Wash Plate 4	7	Standard	Wash Solution 2	150 µL
Elution Plate	8	Standard	Elution Buffer	50 µL
Tip Comb	1	Deep Well	Place a KingFisher™ 96 tip comb for deep-well magnets into a KingFisher™ 96 Deep-Well 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

1. Ensure that the instrument is set up for processing with the deep well magnetic head and select the program on the instrument.

   Program: **MagMAX_mirVana_Biofluids**
2. Start the run and load the prepared processing plates in their positions when prompted by the instrument (see “Set up the processing plates” on page 4).
3. Load the sample plate (containing lysate, isopropanol, and Binding Beads Mix) when prompted by the instrument.
4. When prompted by the instrument (30–35 minutes after the initial start):
   a. Remove the DNase Plate from the instrument.
   b. 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.
   c. Load the DNase Plate back onto the instrument, and press **Start**.
5. 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, before using the RNA in downstream applications, place the Elution Plate on the Magnetic Stand-96, then transfer eluates to a fresh Elution Plate.

   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

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

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

[1] Setting for Lab-Line™ shaker.

6. 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

1. 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.

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	1050 rpm (Speed 8) [1]

[1] Setting for Lab-Line™ shaker.

5. Add 135 µL of isopropanol.

3 Set up the processing plate

Add processing reagents as indicated in the following table.

Table 4 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

1. 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.
2. Start the run and load the prepared processing plate when prompted by the instrument (see Table 4).
3. When prompted by the instrument (approximately 30–35 minutes after initial start):
   a. Remove the plate from the instrument.
   b. 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.

   c. Load the plate back onto the instrument, and press **Start**.
4. 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.
5. 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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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0011137 E.0

Revision	Date	Description
E.0	2 October 2023	Step added to section for sample digestion with Proteinase K.
D.0	16 June 2022	The volumes for blood samples and PK Digestion Buffer were updated in the protocol for the KingFisher™ Apex Purification System.
C.0	19 April 2021	Support was added for the KingFisher™ Apex Purification System.
B.0	11 December 2018	The centrifugation speeds were updated.
A.0	11 May 2015	New document for the MagMAX™ *mir*Vana™ Total RNA Isolation Kit.

The information in this guide is subject to change without notice.

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