

MagMAX™ mirVana™ Total RNA Isolation Kit

High-throughput isolation of RNA (including small RNA) from cells

Catalog Number A27828

Pub. No. MAN0011138 Rev. E



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](https://www.thermofisher.com/support).

Product description

The Applied Biosystems™ MagMAX™ mirVana™ Total RNA Isolation Kit (Cat. No. [A27828](#)) 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 cells. It is optimized for use with the MagMAX™ Express-96 Deep Well Magnetic Particle Processor, the KingFisher™ Flex Purification System with 96 Deep-Well Head (96-well deep well setting), the KingFisher™ Apex Purification System with 96 Deep-Well Head, and the KingFisher™ Duo Prime Purification System (12-well deep well setting).

Contents and storage

Table 1 MagMAX™ mirVana™ Total RNA Isolation Kit (Cat. No. [A27828](#), 96 reactions)

Contents	Amount	Storage
Box 1 of 2		
Proteinase K ^[1]	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	15–25°C
PK Digestion Buffer ^[1]	4.4 mL	
RNA Binding Beads ^[2]	2 mL	
Wash Solution 1 Concentrate ^[3]	20 mL	
Wash Solution 2 Concentrate ^[3]	60 mL	
Rebinding Buffer	4.8 mL	
MagMAX™ TURBO DNase™ Buffer	4.8 mL	
Elution Buffer	9.6 mL	
Processing Plate ^[1]	1	
Elution Plates	2	
Plate Covers	4	

^[1] Not used for RNA isolation from cells.

^[2] Do not freeze the RNA Binding Beads.

^[3] Final volume; see “Prepare wash solutions” on page 2.

Required materials not supplied

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com). MLS: Fisher Scientific ([fisherscientific.com](https://www.fisherscientific.com)) or other major laboratory supplier.

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

Item	Source
Instrument, one of the following:	
MagMAX™ Express-96 Deep Well Magnetic Particle Processor	— ^[1]
KingFisher™ Flex Purification System with 96 Deep-Well Head ^[2]	5400630
KingFisher™ Apex Purification System with 96 Deep-Well Head ^[2]	5400930
KingFisher™ Duo Prime Purification System ^[2]	5400110
Other equipment	
Thermo Scientific™ Digital Microplate Shaker, or equivalent titer shaker	88882005
Vortex mixer	MLS
Incubator capable of reaching 65°C with slatted shelves and thermometer	MLS
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Ice bucket	MLS
(Optional) Magnetic Stand-96	AM10027
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 instrument 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
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, then install the program on the instrument” on page 2.

^[3] KingFisher™ Duo Combi Pack (Cat. No. [97003530](#)) includes plates and combs for the KingFisher™ Duo Prime instrument.

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

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.

We recommend the methods described in this user guide to prepare cells from adherent and liquid cultures.

- See “Lyse the cells, then bind the RNA to the RNA Binding Beads” on page 2 if you are using the MagMAX™ Express-96 or the KingFisher™ Flex instrument.
- See “Lyse the cells, then bind the RNA to the RNA Binding Beads” on page 5 if you are using the KingFisher™ Duo Prime instrument.

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™ Digital Microplate Shaker, ensure 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 temperature before use.

Before first use of the kit

(If needed) Download, then install the program on the instrument

The appropriate program for the MagMAX™ mirVana™ Total RNA Isolation Kit must be installed on the instrument before first use.

Isolate RNA from cells

Isolate RNA using the MagMAX™ Express-96 or KingFisher™ Flex instrument

1 Lyse the cells, then 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 using one of the following methods:

- **For adherent cells:** Remove the media from the wells of a 96-well or 24-well culture plate containing up to 1×10^6 cells, then add 200 µL of Lysis Binding Mix to each sample.
- **For suspension cells:** Centrifuge the cells (up to 1×10^6) in a 96-well or 24-well culture plate at $1000 \times g$ for 4 minutes at 4°C, remove the media from the wells, then 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 a KingFisher™ 96 Deep-Well Plate.

1. On the MagMAX™ mirVana™ Total RNA Isolation Kit web page (at thermofisher.com, Cat. No. A27828), go to the **Software & Data Analysis** section.
2. Download the appropriate program for your instrument.

Table 2 Recommended programs

Instrument	Program name
MagMAX™ Express-96	AM1830DW
KingFisher™ Flex	A27828_FLEX_Tissue_Cells
KingFisher™ Apex	A27828_MagMAX_mirVana_TissueCells
KingFisher™ Duo Prime	A27828_DUO_Tissue_Cells

3. See your instrument user guide for instructions for installing the program.

Prepare wash solutions

1. Add 10 mL of isopropanol to Wash Solution 1 Concentrate, then mix.
2. Add 48 mL of ethanol to Wash Solution 2 Concentrate, then mix.

Store the prepared wash solutions at room temperature.

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

- Prepare the TURBO DNase™ Solution as indicated in the following table, mix, then 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, then 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

1 (continued)

- Cover, then shake the plate as indicated.

Time	Speed
5 minutes	1,050 rpm (Speed 8)

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

- Remove the plate from the shaker, then add 20 µL of Binding Beads Mix to each sample.
- Proceed directly to process samples on the instrument (see “Process samples on the instrument” on page 3).

2 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	2	Standard	Wash Solution 1	150 µL
Wash Plate 2	3	Standard	Wash Solution 2	150 µL
DNase Plate ^[2]	4	Standard	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	50–100 µL ^[3]
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.

^[3] Use 50 µL for low input or 100 µL for high input.

3 Process samples on the instrument

Ensure that the instrument is set up with the deep well magnetic head.

- Select the appropriate program on the instrument (see “(If needed) Download, then install the program on the instrument” on page 2).
- Start the run, then load the prepared processing plates in their positions when prompted by the instrument (see Table 3).
- Load the sample plate (containing the lysate, isopropanol, and the Binding Beads Mix) at position 1 when prompted by the instrument.
- When prompted by the instrument (30–35 minutes after the initial start):
 - Remove the DNase Plate from the instrument.
 - Add 50 µL of Rebinding Buffer and 100 µL of isopropanol separately 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 premix the Rebinding Buffer and isopropanol. Add them separately to the samples.

- Load the DNase Plate back onto the instrument, then tap **Start**.
- At the end of the run (about 45 minutes after the initial start), remove the Elution Plate from the instrument, then seal immediately with a new MicroAmp™ Clear Adhesive Film.
 - (Optional) Transfer the eluates to a storage plate after collection.
 - If excess bead residue is seen in the wells, place the Elution Plate on a Magnetic Stand-96 (Cat. No. [AM10027](#)) to capture any residue, then transfer the eluates to a new Elution Plate before using the RNA in downstream applications.

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 instrument

1 Lyse the cells, then bind the RNA to the RNA Binding Beads

- 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

- Collect the cells using one of the following methods:
 - For adherent cells:** Remove the media from the wells of a 96-well or 24-well culture plate containing up to 1×10^6 cells, then add 200 μ L of Lysis Binding Mix to each sample.
 - For suspension cells:** Centrifuge the cells (up to 1×10^6) in a 96-well or 24-well culture plate at $1000 \times g$ for 4 minutes at 4°C, remove the media from the wells, then add 200 μ L of Lysis Binding Mix to each sample.

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

- Lyse the samples by pipetting up and down 5 times.
- Incubate for 5 minutes, then transfer the cell lysates from the cell culture plate to a KingFisher™ 96 Deep-Well Plate.
- Cover, then shake the plate as indicated.

Time	Speed
5 minutes	1,050 rpm (Speed 8)

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

- Remove the plate from the shaker, then add 20 μ L of Binding Beads Mix to each sample.
- Proceed directly to process samples on the instrument (see “Process samples on the instrument” on page 4).

2 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 4 Processing plates

Plate ID	Plate position ^[1]	Plate type	Reagent	Volume per well
Wash Plate 1	3	Standard	Wash Solution 1	150 μ L
Wash Plate 2	4	Standard	Wash Solution 2	150 μ L
DNase Plate ^[2]	5	Deep Well	TURBO DNase™ Solution	50 μ L
Wash Plate 3	6	Standard	Wash Solution 2	150 μ L
Wash Plate 4	7	Standard	Wash Solution 2	150 μ L
Elution Plate	8	Standard	Elution Buffer	50–100 μ L ^[3]
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 μ L of Rebinding Buffer and 100 μ L of isopropanol to the DNase Plate after the DNase treatment step.

^[3] Use 50 μ L for low input or 100 μ L for high input.

3 Process samples on the instrument

Ensure that the instrument is set up with the deep well magnetic head.

- Select the appropriate program on the instrument (see “(If needed) Download, then install the program on the instrument” on page 2).
- Start the run, then load the prepared processing plates in their positions when prompted by the instrument (see Table 4).
- Load the sample plate (containing the lysate, isopropanol, and Binding Beads Mix) when prompted by the instrument.
- When prompted by the instrument (30–35 minutes after the initial start):
 - Remove the DNase Plate from the instrument.
 - Add 50 μ L of Rebinding Buffer and 100 μ L of isopropanol separately 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 premix the Rebinding Buffer and isopropanol. Add them separately to the samples.

- Load the DNase Plate back onto the instrument, then tap **Start**.
- At the end of the run (about 45 minutes after the initial start), remove the Elution Plate from the instrument, then seal immediately with a new MicroAmp™ Clear Adhesive Film.
 - (Optional) Transfer the eluates to a storage plate after collection.
 - If excess bead residue is seen in the wells, place the Elution Plate on a Magnetic Stand-96 (Cat. No. [AM10027](#)) to capture any residue, then transfer the eluates to a new Elution Plate before using the RNA in downstream applications.

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.

1 Lyse the cells, then 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 using one of the following methods:

- **For adherent cells:** Remove the media from the wells of a 96-well or 24-well culture plate containing up to 1×10^6 cells, then add 200 µL of Lysis Binding Mix to each sample.
- **For suspension cells:** Centrifuge the cells (up to 1×10^6) in a 96-well or 24-well culture plate at $1000 \times g$ for 4 minutes at 4°C, remove the media from the wells, then 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 Row H of a KingFisher™ 96 Deep-Well Plate.
5. Cover, then shake the plate as indicated.

Time	Speed
5 minutes	1,050 rpm (Speed 8)

6. Remove the plate from the shaker, then add 20 µL of Binding Beads Mix to each sample.

2 Set up the processing plate

Add processing reagents as indicated in the following table.

Table 5 Volume of processing reagents and plate location

Row ID	Plate row ^[1]	Reagent	Volume per well
Elution	A	Elution Buffer	50–100 µL ^[2]
Tip Comb	B	Place a KingFisher™ Duo 12-Tip Comb in Row B.	
Wash 4	C	Wash Solution 2	150 µL
Wash 3	D	Wash Solution 2	150 µL
DNase ^[3]	E	TURBO DNase™ Solution	50 µL
Wash 2	F	Wash Solution 2	150 µL
Wash 1	G	Wash Solution 1	150 µL

^[1] Row on the MagMAX™ Express-96 Deep Well Plate.

^[2] Use 50 µL for low input or 100 µL for high input.

^[3] 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.

3 Process samples on the instrument

Ensure that the instrument is set up with the deep well 96-well plates.

1. Select the appropriate program on the instrument (see “(If needed) Download, then install the program on the instrument” on page 2).
2. Start the run, then load the prepared processing plate when prompted by the instrument (see Table 5).
3. When prompted by the instrument (30–35 minutes after the initial start):
 - a. Remove the plate from the instrument.
 - b. Add 50 µL of Rebinding Buffer and 100 µL of isopropanol separately 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 premix the Rebinding Buffer and isopropanol. Add them separately to the samples.

- c. Load the plate back onto the instrument, then tap **Start**.
4. At the end of the run (about 45 minutes after the initial start), remove the Elution Plate from the instrument, then 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. MAN0011138 E

Revision	Date	Description
E	13 September 2024	- The following updates were made to the contents and storage table. - The volume for the MagMAX™ TURBO DNase™ Buffer was changed from 4.6 mL to 4.8 mL. - The Proteinase K concentration was removed. - Copy edits and formatting changes were made to align with current documentation style (throughout the document).
D.0	9 August 2022	The automated protocol for the KingFisher™ Apex Purification System was updated.
C.0	19 April 2021	Support was added for the KingFisher™ Apex Purification System.
B.0	14 November 2018	The centrifugation speeds were updated.
A.0	14 May 2015	Initial release.

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

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