MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kit

Manual and automated high-throughput nucleic acid isolation from filtered 50–500 mL wastewater samples

Catalog Numbers A52606

Pub. No. MAN0026482 Rev. A.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 Applied Biosystems™ MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kit (Cat. No. A52606) is developed for rapid isolation of high-quality total nucleic acid (RNA and DNA) from wastewater samples. Nucleic acid that is purified with this kit can be used in a broad range of molecular biology downstream applications, such as sequencing, real-time PCR, and digital PCR. This protocol guides users through manual and automated isolation of RNA and DNA from filtered wastewater samples. Automated nucleic acid isolation is performed using one of the following instruments: KingFisher™ Flex, KingFisher™ Apex, or KingFisher™ Duo Prime.

Contents and storage

The MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kit (Cat. No. A52606) contains reagents sufficient for 20 reactions.

Component	Amount	Storage
Lysis Buffer	80 mL	15°C to 30°C
Binding Solution	50 mL	
Wash Buffer	200 mL	
Elution Solution	25 mL	
Proteinase K	4 mL	
DNA/RNA Binding Beads (Binding Beads)	2 mL	

Additional reagents can be ordered separately: Lysis Buffer (Cat. No. A42361), Binding Solution (Cat. No. A42359), Wash Solution (Cat. No. A42360), Proteinase K (Cat. No. A42363), and Binding Beads (Cat. No. A42362).

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or another major laboratory supplier.

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

Table 1 Materials required for all sample preparation and isolation methods (manual and automated).

Item	Source
Equipment	
Standard laboratory mixer (vortex or equivalent)	MLS
Centrifuge for 50-mL conical tubes (10,000 × *g*)	MLS
Benchtop centrifuge	MLS
Adjustable micropipettors	MLS
Multichannel micropipettors	MLS
Incubator set at 65°C[1]	MLS
Consumables	
Nalgene™ Rapid-Flow™ Sterile Single Use Vacuum Filter Units (nylon; 150-mL volume upper)	150-0045
Nalgene™ Rapid-Flow™ Sterile Single Use Vacuum Filter Units (nylon; 1,000-mL volume upper)	154-0045
Conical Tubes (15 mL)	AM12500
Conical Tubes (50 mL)	AM12501
Nonstick, RNase-Free Microfuge Tubes, 1.5 mL	AM12450
Nonstick, RNase-Free Microfuge Tubes, 2.0 mL	AM12475
MicroAmp™ Clear Adhesive Film	4306311
Reagents	
Ethanol, 100% (molecular biology grade)	MLS
Nuclease-free water	AM9932

[1] Required for heat inactivation.

Table 2 Additional materials required for automated isolation methods.

Item	Source
Instrument, one of the following:	
KingFisher™ Flex Purification System with 96 deep well head	5400630
KingFisher™ Apex Purification System with 96 deep well head	5400930
KingFisher™ Duo Prime Purification System	5400110
Consumables	
KingFisher™ Flex with 24 deep well plate	95040470
KingFisher™ 24 deep well tip comb and plate	97002610
Consumables for the KingFisher™ Flex instrument: • KingFisher™ 96 KF microplate[1] • KingFisher™ 96 tip comb for deep well magnets	 97002540 97002534
Equipment	
KingFisher™ Flex Purification System with 24 deep well head	24074440
Reagent reservoirs	MLS

[1] For tip comb placement, elution plate, and/or storage of eluate.

Table 3 Additional materials required for manual isolation methods.

Item	Source
Plate shaker	MLS
Magnetic stand, one of the following: • DynaMag™-2 Magnet (for tubes) • Magnetic Stand-96 (for 96 deep well plates)	 12321D AM10027
Incubator set at 75°C	MLS

General guidelines

- Perform all steps at room temperature (20–30°C), unless otherwise noted.
- Clean the work surfaces with RNase*Zap*™ to remove nucleases, then wipe the surfaces with 70% to 100% molecular biology grade ethanol to remove additional contaminants.
- Precipitates can form in the Lysis Buffer, Binding Solution, and Wash Buffer if stored below 20°C. If this occurs, warm the reagents at 37°C, then gently mix to dissolve the precipitates. Avoid creating bubbles.

Guidelines for wastewater samples

- Heat-inactivate the wastewater samples upon receival. Heating at 65°C for 30 minutes is typically sufficient for inactivation of SARS-CoV2 and other viral targets in wastewater.

  Note: Longer incubation may be necessary for large wastewater volumes.

Guidelines for Binding Bead Mix

- Vortex Binding Beads thoroughly before each use.
- Ensure that the beads stay fully mixed within the solution during pipetting.
- Avoid creating bubbles during mixing and aliquoting.
- Binding Bead Mix is very viscous so pipet carefully to ensure that the correct volume is added to the sample.

Before first use of the kit

Prepare reagents

Prepare 80% ethanol using 100% absolute ethanol and nuclease-free water.

Prepare 80% ethanol sufficient for a minimum volume of 2 mL per sample.

Download and install the program (automated methods)

The appropriate program for the kit must be installed on the instrument before first use.

1. On the MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kit (Cat. No. A52606) product web page, navigate to the **Product Literature** section. Right-click the appropriate file to download the latest version of the program for your instrument.

   Note: If you are using the KingFisher™ Apex instrument, download the program from the KingFisher™ Apex Protocol Library directly from the instrument.

Instrument	Program name
KingFisher™ Flex	MagMAX_Wastewater_Flex96_V2.bdz
KingFisher™ Apex	MagMAX_Wastewater_Apex96_for_Apex.bdz
KingFisher™ Duo Prime	MagMAX_Wastewater_DUO96.bdz

2. See your instrument user guide or contact Technical Support for instructions for installing the program.

Before each use of the kit

1. Vortex the Binding Beads vigorously to ensure that the beads are fully resuspended.

2. Prepare Binding Bead Mix—Combine the following components for the required number of samples, plus 10% overage.

Component	Volume per well (24 deep-well plates)
Binding Solution	2,000 µL
Binding Beads	100 µL
Total Binding Bead Mix	**2,100 µL**

3. Mix well by inversion, then store at room temperature.

Prepare samples for isolation

Prepare 50–500 mL wastewater samples

1. Transfer the wastewater sample to multiple 50-mL conical tubes.

   Example:
   - To process 150 mL of clear supernatant—Divide 170 mL of sample into four tubes.
   - To process 500 mL of clear supernatant—Divide 550 mL of sample into eleven tubes.

2. *(Optional)* Spike the sample with the appropriate volume of viral or pathogen target as required per sample for your experiment.

3. Centrifuge at 10,000 × *g* (deceleration speed set at 5) for 10 minutes.

   IMPORTANT! Centrifugation is recommended to remove inhibitors of PCR or RT-PCR. It is critical to obtain a clear supernatant.

4. Carefully collect the clear supernatant from each tube. Leave 5 mL at the bottom of the tube to avoid disturbing the pellet.

   Example: For four tubes, collect 150 mL of supernatant, leaving 5 mL at the bottom of each tube.

5. Vacuum-filter the supernatant using the appropriate nylon Nalgene™ Rapid-Flow™ filter unit as indicated.

Sample volume	Recommended filter unit
50–150 mL	Cat. No. 150-0045
500 mL	Cat. No. 154-0045

6. Using a razor blade, cut a line down the center of the filter membrane, then transfer the membrane to the lid of the filter unit with forceps.

7. Finely mince the membrane on the lid of the filter unit using the razor blade and forceps.

8. Transfer the membrane pieces to a 15-mL conical tube, then add the following components in the order indicated.

Component	Volume per sample	
	For 50–150 mL initial sample volume	For 500 mL initial sample volume
Lysis Buffer	2 mL	2.5 mL
Vortex at high speed for 1 minute, then invert the tube and vortex upside down for an additional minute.		
Proteinase K	160 µL	200 µL

9. Incubate for 20 minutes at 65°C.

10. Centrifuge at 4,000 × *g* for 2 minutes.

11. Transfer the entire volume of supernatant to the appropriate well of a new 24 deep-well plate (Sample Plate).

    Note: Press down on the membrane pieces with the pipette tip to collect all of the supernatant.

Isolate SARS-CoV2 or other viral nucleic acid with the KingFisher™ Flex or KingFisher™ Apex instrument

Note: Samples must be prepared before starting this procedure. See "Prepare samples for isolation" on page 3.

1 Set up the instrument

1.1. Ensure that the instrument is set up with the proper magnetic head and heat block, as indicated in the following table:

Component	Type
Magnetic head	24 deep-well magnetic head
Heat block	24 deep-well heating block

IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields and potential harm to the instrument.

1.2. Ensure that the appropriate program has been downloaded and installed on the instrument (see "Download and install the program (automated methods)" on page 2).

2 Set up the processing plates

2.1. Set up the Wash, Elution, and Tip Comb Plates outside of the instrument according to the following table:

Plate ID	Plate position	Plate type	Reagent	Volume per well	
				For ≤170 mL initial sample volume	For 550 mL initial sample volume
Wash 1 Plate	2	24 deep-well	Wash Buffer	4,000 µL	
Wash 2 Plate	3	24 deep-well	80% Ethanol	4,000 µL	
Elution Plate	4	24 deep-well	Elution Solution	100 µL[1,2]	200 µL[1]
Tip Comb	5	Place the 24 deep-well tip comb in a standard 24 deep-well plate			

[1] We recommend an elution volume of 100 µL, however, 50 µL can be used if a more concentrated sample is needed for downstream analysis.

[2] If leftover beads are observed in the Elution Plate, the beads can be separated by putting the Elution Plate on a 96-well magnet stand.

Note: If a 200-µL eluate appears cloudy, a 1:10 dilution can be used for downstream analysis.

2.2. *(Optional)* To prevent evaporation and contamination, cover the prepared processing plates with paraffin film or MicroAmp™ Clear Adhesive Film until they are loaded into the instrument.

3 Combine prepared samples with the Binding Bead Mix

Invert the tube of Binding Bead Mix several times to resuspend the beads, then add 2,100 µL of the Binding Bead Mix to each sample.

Note:

- Keep the Binding Bead Mix thoroughly mixed throughout the pipetting procedure.
- Pipet slowly to ensure the correct volume of Binding Bead Mix is added to each well.
- DO NOT reuse pipette tips to add the Binding Bead Mix to the samples, as the high viscosity will cause variations in the volumes added.

4 Process samples on the instrument

4.1. Select the appropriate program on the instrument (see "Download and install the program (automated methods)" on page 2).

4.2. Start the run, then load the prepared Sample Plate (plate position 1) and processing plates into position when prompted by the instrument.

4.3. At the end of the run (~29 minutes), immediately remove the Elution Plate from the instrument, then cover the plate. Alternatively, transfer the eluate to a new tube or plate for final storage.

The isolated nucleic acid is ready for immediate use.

4 Process samples on the instrument *(continued)*

Store the isolated nucleic acid at –20°C for up to 6 months or at –80°C for greater than 6 months. Isolated nucleic acid can be stored in 1.5-mL tubes.

Isolate SARS-CoV2 or other viral nucleic acid with the KingFisher™ Duo Prime instrument

Note: Samples must be prepared before starting this procedure. See "Prepare samples for isolation" on page 3.

1 Set up the instrument

1.1. Ensure that the instrument is set up with the proper magnetic head and heat block, as indicated in the following table:

Component	Type
Magnetic head	6-pin magnetic head
Heat block	6-well heating block (for 24 deep-well plates)

IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields and potential harm to the instrument.

1.2. Ensure that the appropriate program has been downloaded and installed on the instrument (see "Download and install the program (automated methods)" on page 2).

2 Set up the processing plates

2.1. Set up the Wash, Elution, and Tip Comb Plates outside of the instrument according to the following table:

Row ID	Plate row	Plate type	Reagent	Volume per well	
				For ≤170 mL initial sample volume	For 550 mL initial sample volume
Sample	A	24 deep-well	Sample lysate + Proteinase K + Binding Bead Mix	~4,200 µL	
Tip Comb	B		Place a tip comb in the plate		
Wash 1	C		Wash Buffer	4,000 µL	
Wash 2	D		80% Ethanol	4,000 µL	
Elution	Separate tube strip		Elution Solution	100 µL[1,2]	200 µL

[1] We recommend an elution volume of 100 µL, however, 50 µL can be used if a more concentrated sample is needed for downstream analysis.

[2] If leftover beads are observed in the Elution Plate, the beads can be separated by putting the Elution Plate on a 96-well magnet stand.

Note: If a 200-µL eluate appears cloudy, a 1:10 dilution can be used for downstream analysis.

2.2. *(Optional)* To prevent evaporation and contamination, cover the prepared processing plates with paraffin film or MicroAmp™ Clear Adhesive Film until they are loaded into the instrument.

3 Combine prepared samples with the Binding Bead Mix

Invert the tube of Binding Bead Mix several times to resuspend the beads, then add 2,100 µL of the Binding Bead Mix to each sample in Row A of the Sample Plate.

Note:

- Keep the Binding Bead Mix thoroughly mixed throughout the pipetting procedure.
- Pipet slowly to ensure the correct volume of Binding Bead Mix is added to each well.
- DO NOT reuse pipette tips to add the Binding Bead Mix to the samples, as the high viscosity will cause variations in the volumes added.

4	Process samples on the instrument	**4.1.** Select the appropriate program on the instrument (see “Download and install the program (automated methods)” on page 2). **4.2.** Start the run, then load the prepared plates into position when prompted by the instrument. **4.3.** At the end of the run (~28 minutes), immediately remove the plate from the instrument, then transfer the eluate to a new tube or plate for final storage. The isolated nucleic acid is ready for immediate use. Store the isolated nucleic acid at –20°C for up to 6 months or at –80°C for greater than 6 months. Isolated nucleic acid can be stored in 1.5-mL tubes.

Isolate SARS-CoV2 or other viral nucleic acid (manual method)

Note: Samples must be prepared before starting this procedure. See “Prepare samples for isolation” on page 3.

1	Combine prepared samples with the BInding Bead Mix	**1.1.** Invert the tube of Binding Bead Mix several times to resuspend the beads, then add 2,100 µL of Binding Bead Mix to each sample. **Note:** • Keep the Binding Bead Mix thoroughly mixed throughout the pipetting procedure. • Pipet slowly to ensure the correct volume of Binding Bead Mix is added to each well. • DO NOT reuse pipette tips to add the Binding Bead Mix to the samples, as the high viscosity will cause variations in the volumes added. **1.2.** Seal the plate with MicroAmp™ Clear Adhesive Film. Apply firm pressure to the adhesive film during application to ensure a tight, leak-proof seal. The plate is properly sealed when an imprint of each well is visible on the surface of the film. **1.3.** Shake the sealed plate at 900 rpm for 5 minutes. **1.4.** Incubate the plate at 65°C for 20 minutes. **1.5.** Place the plate on a magnetic stand for at least 5 minutes, or until all of the beads have collected.
2	Wash the beads	**2.1.** With the plate on the magnetic stand, carefully remove the adhesive film, then discard the supernatant. **IMPORTANT!** Avoid disturbing the beads. **2.2.** Remove the plate from the magnetic stand, then add 4 mL of Wash Buffer to each sample. **2.3.** Reseal the plate, then shake at 800 rpm for 30 seconds. **2.4.** Place the plate on the magnetic stand for 3–5 minutes, or until all of the beads have collected at the bottom of the plate. **2.5.** With the plate on the magnetic stand, carefully remove the adhesive film, then discard the supernatant. **IMPORTANT!** Avoid disturbing the beads. **2.6.** Repeat step 2.2 through step 2.5, using 4 mL of 80% ethanol. **2.7.** Shake the plate at 800 rpm for 3 minutes to dry the beads.

3 Elute the nucleic acid

3.1. Add the appropriate volume of Elution Solution to each sample based on the initial sample volume.

Initial sample volume	Volume of Elution Solution per well
≤170 mL	100 µL[1]
550 mL	200 µL

[1] We recommend an elution volume of 100 µL, however, 50 µL can be used if a more concentrated sample is needed for downstream analysis.

Note: If a 200-µL eluate appears cloudy, a 1:10 dilution can be used for downstream analysis.

3.2. Seal the plate with MicroAmp™ Clear Adhesive Film, then incubate at 75°C for 5 minutes.

3.3. Shake at 800 rpm for 5 minutes.

3.4. Place the plate on the magnetic stand for 3–5 minutes, or until all of the beads have collected at the bottom of the plate.

3.5. With the plate on the magnetic stand, carefully remove the adhesive film, then transfer the eluates to a new standard plate (not a deep-well plate).

IMPORTANT! Immediately seal the plate containing the eluate to prevent evaporation.

The isolated nucleic acid is ready for immediate use.

Store the isolated nucleic acid at –20°C for up to 6 months or at –80°C for greater than 6 months.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision	Date	Description
A.0	11 March 2022	New document created for introduction of MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kit with emphasis on the manual and automated high-througput nucleic acid isolation from filtered wastewater samples.