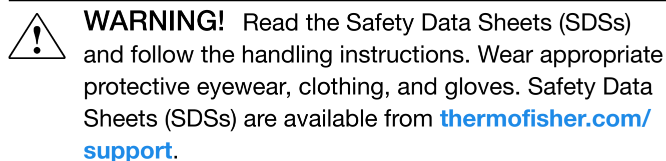
applied biosystems USER GUIDE

MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kit

Manual and automated high-throughput nucleic acid isolation from 200 µL pre-concentrated wastewater samples

Catalog Numbers A52606

Pub. No. MAN0025693 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 scalable, 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 200 µL pre-concentrated 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 100 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), Elution Solution (Cat. No. A42364), 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
Benchtop centrifuge	MLS
Adjustable micropipettors	MLS
Multichannel micropipettors	MLS
Consumables	
KingFisher™ 96 Deep-Well Plate	95040450
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

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

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Item	Source
Consumables	
KingFisher™ 96 Deep-Well Plate	95040450
KingFisher™ 96 tip comb for deep-well magnets	97002534
KingFisher™ 96 KF microplate (for the KingFisher™ Flex instrument only)	97002540
Equipment	
Reagent reservoirs	MLS

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 at a room temperature that is too cold. 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 sufficient solution 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.kfx
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 (96 deep-well plates)
Binding Solution	500 µL
Binding Beads	20 µL
Total Binding Bead Mix	**520 µL**

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

Prepare samples for isolation (all methods)

1. Add the following components to a 2-mL tube in the order indicated:
   - Wastewater sample—200 µL
   - *(Optional)* Viral or pathogen spike—as required per sample for your experiment
   - Lysis Buffer—200 µL
2. Vortex at high speed (setting 10) for 10 seconds.
3. Centrifuge at 10,000 × *g* for 1–2 minutes.
4. Transfer the supernatant (400 µL, plus the spike volume if used) to the appropriate wells of a new 96 deep-well plate (Sample Plate).

   IMPORTANT! Avoid disturbing the pellet.

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 (all methods)" 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	96 deep-well magnetic head
Heat block	96 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
Wash 1 Plate	2	96 deep-well	Wash Buffer	1,000 µL
Wash 2 Plate	3	96 deep-well	80% Ethanol	1,000 µL
Elution Plate	4	96 deep-well	Elution Solution	50–100 µL[1,2]
Tip Comb	5	Place the 96 deep-well tip comb in a standard 96 deep-well plate		

[1] An elution volume of 100 µL can be used if the larger volume 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.

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 samples with Proteinase K and the Binding Bead Mix

3.1. Add 40 µL of Proteinase K to each sample in the Sample Plate.

3.2. Invert the tube of Binding Bead Mix several times to resuspend the beads, then add 520 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 (~28 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. Store the isolated nucleic acid at –20°C for up to 6 months or at –80°C for greater than 6 months.

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 (all methods)" 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	12-pin magnetic head
Heat block	12-well heating block (for 96 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	Reagent	Volume per well
Sample	A	Sample lysate + Proteinase K + Binding Bead Mix	960 µL
Tip Comb	B	Place a tip comb in the plate	
	C	*Empty*	
Wash 1	D	Wash Buffer	1,000 µL
Wash 2	E	80% Ethanol	1,000 µL
Elution	Separate tube strip	Elution Solution	50 µL–100 µL

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 samples with Proteinase K and the Binding Bead Mix

3.1. Add 40 µL of Proteinase K to each sample in the Sample Plate.

3.2. Invert the tube of Binding Bead Mix several times to resuspend the beads, then add 520 µL of the Binding Bead Mix to each sample in Row H 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.

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

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

1	Combine samples with Proteinase K and the Binding Bead Mix	**1.1.** Add 40 µL of Proteinase K to each sample in the Sample Plate. **1.2.** Invert the tube of Binding Bead Mix several times to resuspend the beads, then add 520 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. **1.3.** 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. **1.4.** Shake the sealed plate at 900 rpm for 5 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 1 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 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 2b through step 2e, using 1 mL of 80% ethanol. **2.7.** Shake the plate at 800 rpm for 2 minutes to dry the beads.

3	Elute the nucleic acid	

3.1. Add 50–100 µL of Elution Solution to each sample, then seal the plate with MicroAmp™ Clear Adhesive Film.

3.2. 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 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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Revision history: Pub. No. MAN0025693

Revision	Date	Description
A.0	14 September 2021	New document created for introduction of MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kit.

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