MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kit

Manual and automated high-throughput nucleic acid isolation from 10 mL wastewater samples, using Dynabeads™ Wastewater Virus Enrichment beads

Catalog Number A52610

Pub. No. MAN0025695 Rev. C.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. A52610) 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 10 mL 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. A52610) 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	
Dynabeads™ Wastewater Virus Enrichment	10 mL	2°C to 8°C

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
Consumables	
KingFisher™ Flex 24 Deep-Well Plate	95040470
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

Table 2 Additional materials required for automated isolation methods

Item	Source
Instrument, one of the following:	
KingFisher™ Flex Purification System with 24 deep-well head	5400640
KingFisher™ Apex Purification System with 24 combi head	5400940
KingFisher™ Duo Prime Purification System	5400110
Consumables	
KingFisher™ 96 Deep-Well Plate	95040450
KingFisher™ Duo Prime 12-tip comb, for use with KingFisher™ 96 deep-well plates	97003500
KingFisher™ Duo Prime 6-tip comb, for use with KingFisher™ 24 deep-well plates	97003510
Equipment	
Reagent reservoirs	MLS
Equipment for the KingFisher™ Flex instrument:	
24 Deep-Well Head for KingFisher™ Flex Purification System	24074440
KingFisher™ Flex 24 Deep-Well Heating Block	24075440
Equipment for the KingFisher™ Apex instrument:	
KingFisher™ Apex 24 Combi Head	24079940
KingFisher™ Apex 24 deep-well heating block	24075940

Table 3 Additional materials required for manual isolation methods

Item	Source
HulaMixer™ Sample Mixer	15920D
Plate shaker	MLS
DynaMag™–50 Magnet	12302D
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 programs for the kit must be installed on the instrument before first use.

1. On the MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kit (Cat. No. A52610) 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 programs from the KingFisher™ Apex Protocol Library directly from the instrument.

Instrument	Program name	
—	Pre-enrichment	Nucleic acid isolation
KingFisher™ Duo Prime	Dyna_Duo24_WastewaterEnrich.bdz	MagMAX_Wastewater_Duo96.bdz
—	Enrichment and isolation combined	
KingFisher™ Flex	MagMAX_Wastewater_10mL_Flex24	
KingFisher™ Apex	MagMAX_Wastewater_10mL_Apex24	

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.

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

Component	Volume per well
Binding Solution	500 µL
Binding Beads	20 µL
Total Binding Bead Mix	**520 µL**

Prepare samples for isolation (all methods)

1. To process one 10 mL sample volume, transfer 15 mL of wastewater to a 50 mL conical tube.

2. *(Optional)* Spike the sample with a viral or pathogen target.

3. Centrifuge at 10,000 × *g* (deceleration speed set at 5) for 10 minutes, then confirm that the supernatant is free of particulates. If particulates are floating in the supernatant, repeat the centrifugation for an additional 5 minutes.

   IMPORTANT! It is critical to obtain a clear supernatant for better sensitivity in downstream analysis. Handle sample tubes carefully to avoid disturbing the pellet. If small particulates can still be seen floating in the supernatant after centrifugation, place the tubes on a rack for 2 minutes, or until the particulates settle, before collecting the supernatant.

4. Carefully transfer 10 mL of the supernatant to a new 50 mL conical tube, then proceed as indicated.

   IMPORTANT! Pipet slowly and leave 5 mL of supernatant at the bottom of the tube to avoid disturbing the pellet.

   - **For automated isolation—**Transfer 4.9 mL and 5 mL of the supernatant to the appropriate wells in each of two new 24 deep-well plates (Sample Plate 1 and Sample Plate 2, respectively). Each well should have the wastewater sample. For example, 4.9 mL of sample should be filled in A1 of Sample Plate 1 and 5 mL of sample in A1 of Sample Plate 2.
   - **For manual isolation—**Transfer 10 mL of the supernatant to a new 50 mL conical tube.

Isolate SARS-CoV2 or other viral nucleic acid with Dynabeads™ Wastewater Virus Enrichment beads and 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.

Workflow

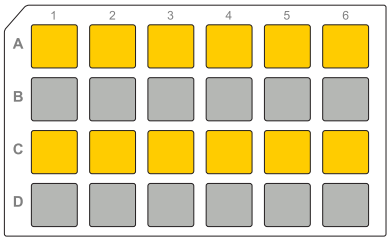
Processing plates

Set up the instrument

Set up with the proper magnetic head, heat block, and program.

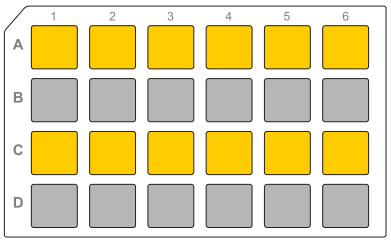
Set up the processing plates (for pre-enrichment): Sample Plate 1

- Rows A and C: Add 4,900 µL of wastewater sample and 100 µL Dynabeads™ Wastewater Virus Enrichment beads.
- Rows B and D: Leave empty.



Set up the processing plates (for pre-enrichment): Sample Plate 2

- Rows A and C: Add 5,000 µL of wastewater sample.
- Rows B and D: Leave empty.

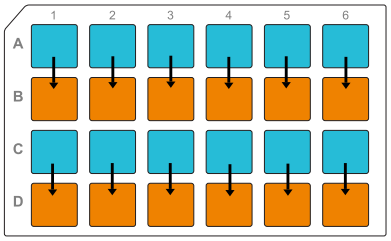


Process samples on the instrument (pre-enrichment)

Start the run, then load the prepared sample and plates.

Transfer eluate, then combine samples with Proteinase K: Elution 1

1. Transfer 500 µL of wastewater sample in Lysis Buffer from row A to row B and from row C to row D.
2. Add 40 µL of Proteinase K to rows B and D.

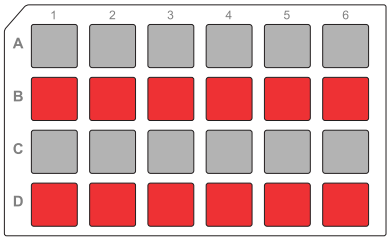


Add Binding Bead Mix, then process samples on the instrument

1. Add 520 µL of Binding Bead Mix to rows B and D. Leave rows A and C empty.
2. Load the plate, then start the run.
3. Cover, then store the isolated nucleic acid.

Note:
The contents per well in Rows B and D of the following plates:

- Wash Buffer 1—1,000 µL of Wash Buffer
- Wash Buffer 2—1,000 µL of 80% ethanol
- Final Elution—100 µL of Elution Solution



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 3).

2 Set up the processing plates (for pre-enrichment)

Note: See "Workflow" on page 4 for plate setup guidance.

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

Plate ID	Plate position	Plate type	Reagent	Row position	Volume per well
Sample Plate 1	1	24 deep-well	Sample supernatant	Row A and Row C	4,900 µL
			Dynabeads™ Wastewater Virus Enrichment beads	Row A and Row C	100 µL
Sample Plate 2	2	24 deep-well	Sample supernatant	Row A and Row C[1]	5,000 µL
Elution 1	3	24 deep-well	Lysis Buffer (DO NOT use Elution Solution)	Row A and Row C[1]	500 µL
Wash Buffer 1	4	24 deep-well	Wash Buffer	Row B and Row D	1,000 µL
Wash Buffer 2	5	24 deep-well	80% Ethanol	Row B and Row D	1,000 µL
Final Elution	6	24 deep-well	Elution Solution	Row B and Row D	100 µL
Tip Comb	7	Place the 24 deep-well tip comb in a standard 24 deep-well plate			

[1] Corresponding with Sample Plate 1; up to 12 samples can be processed in a 24 well plate. 6 samples in Row A and 6 samples in Row C.

Note: Add 100 µL of Dynabeads™ Wastewater Virus Enrichment beads to Sample Plate 1 only.

Do not add Dynabeads™ Wastewater Virus Enrichment beads to Sample Plate 2, since sequential binding occurs during the workflow.

IMPORTANT! Elution 1 (plate 3) must be performed with Lysis Buffer, not Elution Solution. Use of Elution Solution, PBS, or TE buffers for the Elution 1 plate can significantly reduce recovery efficiency and sensitivity.

3 Process samples on the instrument (pre-enrichment)

3.1. Select the appropriate program on the instrument (see "Download and install the program (automated methods)" on page 3).

3.2. Start the run, then load the prepared sample and processing plates into position when prompted by the instrument.

4	Transfer eluate, then combine samples with Proteinase K	**4.1.** At the instrument pause (~20 minutes after starting the run), transfer the concentrated and enriched eluate from row A to row B of the same Elution 1 plate (plate 3). Repeat for row C into row D if processing more samples. **Note:** Transferring the enrichment elution to a different row is critical to accommodate for the unused tip comb in row in the following steps. All enrichment happens in rows A and C and all isolation happens in rows B and D. **4.2.** Add 40 µL of Proteinase K to each enriched Elution 1 sample after aliquoting into rows B and D (plate 3).
5	Add Binding Bead Mix, then process samples on the instrument	**5.1.** 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. **5.2.** Once the Elution 1 plate has Proteinase K and Binding/bead mix added to the enriched volumes in row B (see step 4.1), load the plate, then start the run on the instrument. **5.3.** At the end of the run (~27 minutes; total script length is ~45 minutes), immediately remove the Final Elution plate (plate 6) 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 Dynabeads™ Wastewater Virus Enrichment beads and 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	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 3).

2 Set up the processing plates (for pre-enrichment)

Set up the processing plates outside of the instrument according to the following table:

Row ID	Plate row	Plate type	Reagent	Volume per well
Sample Row 1	A	24 deep-well	Sample supernatant	4,900 µL
			Dynabeads™ Wastewater Virus Enrichment beads	100 µL
Sample Row 2	B	24 deep-well	Sample supernatant	5,000 µL
Elution	C	24 deep-well	Lysis Buffer (DO NOT use Elution Solution)	500 µL
Tip Comb	D	Place the 24 deep-well tip comb in a standard 24 deep-well plate		

Add 100 µL of Dynabeads™ Wastewater Virus Enrichment beads to Sample Row 1 only.

Note: Do not add Dynabeads™ Wastewater Virus Enrichment beads to Sample Row 2, since sequential binding occurs during the workflow.

IMPORTANT! The elution must be performed with Lysis Buffer, not Elution Solution. Use of Elution Solution, PBS, or TE buffers can significantly reduce recovery efficiency and sensitivity.

3 Process samples on the instrument (pre-enrichment)

3.1. Select the appropriate program on the instrument (see "Download and install the program (automated methods)" on page 3).

3.2. Start the run, then load the prepared sample and processing plates into position when prompted by the instrument.

3.3. At the end of the run (~45 minutes), immediately remove the Elution Plate from the instrument, then cover the plate or proceed to the next section with the concentrated eluate.

4 Combine samples with Proteinase K, then set up the processing plates

4.1. Transfer the concentrated eluate to the appropriate wells of a new 96 deep-well plate (Sample Plate).

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

4 Combine samples with Proteinase K, then set up the processing plates *(continued)*

4.3. 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	Concentrated sample eluate + 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

4.4. *(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.

5 Add Binding Bead Mix, then process samples on the instrument

5.1. 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.

5.2. Select the appropriate program on the instrument (see “Download and install the program (automated methods)” on page 3).

5.3. Start the run, then load the prepared plates into position when prompted by the instrument.

5.4. 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 with Dynabeads™ Wastewater Virus Enrichment beads (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 Dynabeads™ Wastewater Virus Enrichment beads

1.1. Transfer 10 mL of the sample supernatant to a new 50-mL conical tube.

1.2. Add 100 µL of Dynabeads™ Wastewater Virus Enrichment beads, then incubate on a HulaMixer™ Sample Mixer for 10 minutes at room temperature.

1.3. Place the tube on a DynaMag™–50 Magnet for 3–5 minutes, or until all of the beads have collected.

1.4. Remove the tube from the DynaMag™–50 Magnet, then discard the supernatant.

1.5. Add 500 µL of Lysis Buffer, then pipet up and down several times to resuspend the beads at the bottom and walls of the tube.

Note: Do not vortex to mix.

1.6. Immediately place the tube back on the DynaMag™–50 Magnet for 3–5 minutes, or until all of the beads have collected.

1.7. Transfer the entire volume of each supernatant (500 µL) to the appropriate wells of a 96 deep-well plate.

2 Add Proteinase K and Binding Bead Mix

2.1. Add 40 µL of Proteinase K to each supernatant (500 µL) in the 96 deep-well plate.

2.2. 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.

2.3. 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.

2.4. Shake the sealed plate at 900 rpm for 5 minutes.

2.5. Place the plate on a magnetic stand for at least 5 minutes, or until all of the beads have collected.

3 Wash the beads

3.1. With the plate on the magnetic stand, carefully remove the adhesive film, then discard the supernatant from each well.

IMPORTANT! Avoid disturbing the beads.

3.2. Remove the plate from the magnetic stand, then add 1 mL of Wash Buffer to each sample.

3.3. Reseal the plate, then shake at 800 rpm for 30 seconds.

3.4. Place the plate back on the magnetic stand for 3 minutes, or until all of the beads have collected.

3 Wash the beads *(continued)*

3.5. With the plate on the magnetic stand, carefully remove the adhesive film, then discard the supernatant from each well.

IMPORTANT! Avoid disturbing the beads.

3.6. Repeat step 3.2 through step 3.5 using 1 mL of 80% ethanol.

3.7. Shake the plate at 800 rpm for 2 minutes (uncovered) to dry the beads.

4 Elute the nucleic acid

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

4.2. Incubate at 75°C for 5 minutes.

4.3. Shake at 800 rpm for 5 minutes.

4.4. Place the plate on the magnetic stand for 3 minutes, or until all of the beads have collected.

4.5. 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

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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

Revision history: Pub. No. MAN0025695

Revision	Date	Description
C.0	17 November 2021	• The required materials not supplied list was updated to include: – KingFisher™ Duo Prime 12-tip comb, for use with KingFisher™ 96 deep-well plates – KingFisher™ Duo Prime 6-tip comb, for use with KingFisher™ 24 deep-well plates – KingFisher™ Flex Purification System with 24 deep-well head – KingFisher™ Apex Purification System with 24 combi head • The program information was updated to include a combined enrichment and isolation program name for KingFisher™ Flex and KingFisher™ Apex. • A workflow for processing plates with the KingFisher™ Flex and KingFisher™ Apex was added. • The processing plates (for pre-enrichment) reference table for the KingFisher™ Flex and KingFisher™ Apex protocol was updated to include row positions and plate IDs for Wash Buffer 1, Wash Buffer 2, and Final Elution. • A step to set up the processing plates for the KingFisher™ Flex and KingFisher™ Apex protocol was removed.
B.0	16 September 2021	Corrected the manual protocol to include the addition of Proteinase K.
A.0	14 September 2021	New document created for introduction of MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kit.