### MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit

High throughput isolation of nucleic acid (RNA and DNA) from wastewater samples Catalog Numbers A42357, A42358

Pub. No. MAN0025535 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<sup>™</sup> MagMAX<sup>™</sup> Microbiome Ultra Nucleic Acid Isolation Kit is developed for scalable, rapid purification of high-quality total nucleic acid (RNA and DNA) from fecal and wastewater samples. You can use the nucleic acid purified with this kit in a broad range of molecular biology downstream applications, such as sequencing and real-time PCR. This protocol guides users through automated isolation of RNA and DNA from wastewater samples using the KingFisher<sup>™</sup> Flex instrument.

### Contents and storage

Reagents that are provided in the kit are sufficient for 40 reactions using 24 deep-well plates and 100 reactions using 96 deep-well plates (Cat. No. A42357 and A42358).

Table 1 Components of MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit

Component	Amount	Storage
Lysis Buffer	80 mL	
Binding Solution	50 mL	
Wash Buffer	200 mL	
Elution Solution	20 mL	1500 + 0500
Proteinase K	4 mL	15°C to 25°C
Total Nucleic Acid Binding Beads	2 mL	
96-Well Bead Beating Plate or Bead Tubes <sup>[1]</sup>	1 plate or 100 tubes	

<sup>[1]</sup> Bead plates or tubes are only required to perform SARS-CoV2 nucleic acid purification from >50 mL wastewater samples using Nalgene™ filters and KingFisher™ Flex.

For 200 reactions using 24 deep-well plates and 1,000 reaction volume using 96 deep-well plates, use Cat. No. A42361 (Lysis Solution), A42359 (Binding Solution), A42360 (Wash Solution), A42364 (Elution Solution), A42363 (Proteinase K), and A42362 (Binding Beads).

For bead tubes and plate sold separately, use Cat. No. A42351 (Bead Tubes, 100), and A42331 (Bead Plate, 1).

### Required materials not supplied

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

Item	Source		
Magnetic particle processor:			
KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head	5400630		
Equipment			
24 Deep-Well Head for KingFisher™ Flex Magnetic Particle Purification System	24074440		
Adjustable micropipettors	MLS		
Multi-channel micropipettors	MLS		
Consumables			
Deep-well plates:			
KingFisher™ 96 Deep-Well Plate	95040450		
KingFisher™ Flex 24 Deep-Well Plate	95040470		
96-well standard plate (for use with KingFisher <sup>™</sup> Flex comb placement, elution plate, and/or eluate storage			
KingFisher™ 96 KF microplate	97002540		
Tip combs, compatible with the magnetic particle processor used:			
KingFisher™ Flex 24 deep-well tip comb and plate	97002610		
KingFisher™ 96 tip comb for DW magnets, for Flex protocol only	97002534		
Reagents			
Ethanol, 100% (molecular biology grade)	MLS		
Nuclease-free water	AM9932		



Item	Source
Materials	
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
Foil seals <sup>[1]</sup>	14-222-342
Reagent reservoirs	MLS
Required for protocols as specified	
10 mL wastewater samples:	
Dynabeads™ Intact Virus Enrichment	10701D
(optimized for SARS-CoV2)	
50 mL wastewater samples:	
Dynabeads™ Intact Virus Enrichment	10701D
(optimized for SARS-CoV2)	107010
HulaMixer™ Sample Mixer	15920D
DynaMag <sup>™</sup> –50 Magnet	12302D
50 mL-250 mL wastewater samples (option 1):	
Nalgene™ Rapid-Flow™ Sterile Single Use Vacuum Filter Units	156-4045
50 mL-250 mL wastewater samples (option 2):	
Intact Virus Precipitation Reagent	10720D
(optimized for SARS-CoV2)	101200

<sup>[1]</sup> Required if you are using a bead-beating plate.

### General guidelines

- Perform all steps at room temperature (20–25°C), unless otherwise noted.
- Clean the work surfaces with RNaseZap<sup>™</sup> to remove nucleases, then wipe the surfaces with 70% to 100% molecular biology grade ethanol to remove additional contaminants.
- Precipitates can occur if the Lysis Buffer or Binding Solution is stored when room temperature is too cold. If there are precipitates, warm the reagents at 37°C and 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 80% Ethanol from 100% absolute Ethanol and nuclease-free water.

Prepare enough solution for a minimum volume of 2 mL per sample.

#### Before each use of the kit

- 1. Vortex beads vigorously to ensure they are homogenous.
- Prepare the Binding Bead Mix according to the following table:

Component	Volume per well <sup>[1]</sup> 96 deep-well plates	Volume per well <sup>[1]</sup> 24 deep-well plates
Binding Solution	500 μL	2,500 µL
Total Nucleic Acid Binding Beads	20 μL	100 µL
Total volume	520 μL	2,600 μL

<sup>[1]</sup> Use 10% overage calculation when making a master mix for use with multiple samples.

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

# Perform SARS-CoV2 (or other viral targets) nucleic acid purification from 1 mL wastewater samples using KingFisher™ Flex

Set up the instrument

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

Component	Туре
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 proper program **MagMAX\_Microbiome\_Flex24\_Wastewater** has been downloaded from the product page and loaded onto the instrument.

2 Set up the processing plates

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	24 deep-well	Wash Buffer	2,500 μL
Wash 2 Plate	3	24 deep-well	Wash Buffer	2,500 μL
Wash 3 Plate	4	24 deep-well	80% Ethanol	2,500 μL
Wash 4 Plate	5	24 deep-well	80% Ethanol	2,500 μL
Elution Plate	6	24 deep-well	Elution Solution	100 µL <sup>[1]</sup>
Tip Comb	7	Place the 24 d	eep-well tip comb in a st plate	andard 24 deep-well

<sup>[1]</sup> Elution volume can be reduced to 50 µL for a more concentrated sample. 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: 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 Lyse, bind, wash, then elute the total nucleic acid

3.1. Transfer up to 1,000  $\mu$ L of the wastewater sample to the appropriate wells of a new 24 deep-well plate. This is the Sample Plate.

Note: 1000  $\mu$ L is the optimal wastewater sample starting volume, however, up to 1,500  $\mu$ L can be used. If using more than 1000  $\mu$ L of the wastewater sample, the Lysis Buffer should be adjusted to a total volume of 2,000  $\mu$ L.

- 3.2. Add 1,000 µL of Lysis Buffer to the wells containing the wastewater sample.
- 3.3. Add 200  $\mu L$  of Proteinase K to the wells containing the wastewater sample.
- 3.4. Invert the Binding Bead Mix to mix, then add 2,600 µL to each sample in the Sample Plate.

**Note:** Binding Bead Mix is viscous, so pipet slowly and mix frequently by inversion to ensure the correct volume and even distribution of beads to all the wells. DO NOT reuse pipette tips to add Binding Bead Mix to the samples, as the high viscosity will cause variations in the volumes added.

3.5. Select the program MagMAX\_Microbiome\_Flex24\_Wastewater on the instrument.

- 3 Lyse, bind, wash, then elute the total nucleic acid (continued)
- **3.6.** Start the run, then load the prepared sample (plate position 1) and processing plates into position when prompted by the instrument.
- **3.7.** After the protocol is complete (~33 minutes after start), immediately remove the Elution Plate from the instrument, then cover the plate or transfer the eluate to the tube or plate of choice for final storage.

# Perform SARS-CoV2 (or other viral targets) nucleic acid purification from 200 μL pre-concentrated wastewater samples using KingFisher<sup>™</sup> Flex

Note: If wastewater samples are pre-concentrated before nucleic acid isolation, the following workflow can be used.

Set up the instrument

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

Component	Туре
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 proper program (**MagMAX\_Microbiome\_Flex96\_Wastewater**) has been downloaded from the product page and loaded onto the instrument.

2 Set up the processing plates

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	Wash Buffer	1,000 µL
Wash 3 Plate	4	96 deep-well	80% Ethanol	1,000 µL
Wash 4 Plate	5	96 deep-well	80% Ethanol	1,000 µL
Elution Plate	6	96 deep-well	Elution Solution	50 μL <sup>[1]</sup>
Tip Comb	7	Place the 96 d	eep-well tip comb in a st plate	andard 96 deep-well

<sup>[1]</sup> 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: To prevent evaporation and contamination, cover the prepared processing plates with paraffin film or MicroAmp $^{\text{TM}}$  Clear Adhesive Film until they are loaded into the instrument.

- Lyse, bind, wash, then elute the total nucleic acid
- 3.1. Transfer 200  $\mu$ L of the wastewater sample to the appropriate wells of a new deep-well plate. This is the Sample Plate.
- 3.2. Add 200 µL of Lysis Buffer to the wells containing the wastewater sample.
- 3.3. Add 40 µL of Proteinase K to the wells containing the wastewater sample.
- 3.4. Invert the Binding Bead Mix to mix, then add 520 μL (500 μL of Binding Solution and 20 μL of Binding Beads) to each sample in the Sample Plate.

**Note:** Binding Bead Mix is viscous, so pipet slowly and mix frequently by inversion to ensure the correct volume and even distribution of beads to all the wells. DO NOT reuse pipette tips to add Binding Bead Mix to the samples, as the high viscosity will cause variations in the volumes added.

3.5. Select the program MagMAX\_Microbiome\_Flex96\_Wastewater on the instrument.

- 3 Lyse, bind, wash, then elute the total nucleic acid (continued)
- **3.6.** Start the run, then load the prepared sample (plate position 1) and processing plates into position when prompted by the instrument.
- **3.7.** After the protocol is complete (~35 minutes after start), immediately remove the Elution Plate from the instrument, then cover the plate or transfer the eluate to the tube or plate of choice for final storage.

# Perform SARS-CoV2 (or other viral targets) nucleic acid purification from 10 mL wastewater samples using Dynabeads<sup>™</sup> Intact Virus Enrichment beads and KingFisher<sup>™</sup> Flex

Set up the instrument

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

Component	Туре
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 proper program **Dyna\_Flex24\_WastewaterEnrich** has been downloaded from the product page and loaded onto the instrument.

2 Set up the sample and processing plates

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	Volume per well
Sample Plate 1	1	24 deep-well	Wastewater + 100 µL Dynabeads <sup>™</sup> Intact Virus Enrichment beads	5,000 µL
Sample Plate 2	2	24 deep-well	Wastewater	5,000 µL
Elution Plate	3	24 deep-well	Lysis Buffer	500 μL
Tip Comb	4	Place the 24 dee	ep-well tip comb in the de with it	eep-well plate provided

- 3 Concentrate the viral particles
- **3.1.** Select the program **Dyna\_Flex24\_WastewaterEnrich** and load the prepared sample and processing plates into position when prompted by the instrument, then start the run.
- **3.2.** After the protocol is complete (~45 minutes after start), immediately remove the Elution Plate from the instrument and cover the plate or continue to the next step.
- 4 Lyse, bind, wash, then elute the total nucleic acid
- **4.1.** Transfer the concentrated eluate from the previous Flex run to the appropriate wells of a new 96 deep-well plate. This is the Sample Plate.
- 4.2. Add 40 µL of Proteinase K to the wells containing the wastewater sample.
- **4.3.** 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	Wash Buffer	1,000 µL
Wash 3 Plate	4	96 deep-well	80% Ethanol	1,000 µL
Wash 4 Plate	5	96 deep-well	80% Ethanol	1,000 µL
Elution Plate	6	96 deep-well	Elution Solution	50 μL
Tip Comb	7	Place the 96 de	ep-well tip comb in a s plate	tandard 96 deep-well

4 Lyse, bind, wash, then elute the total nucleic acid (continued)

4.4. Invert, then add 520 μL of the Binding Bead Mix (500 μL of Binding Solution and 20 μL of Binding Beads) to each well containing the enriched wastewater eluate.

**Note:** Binding Bead Mix is viscous, so pipet slowly and mix frequently by inversion to ensure the correct volume and even distribution of beads to all the wells. DO NOT reuse pipette tips to add Binding Bead Mix to the samples, as the high viscosity will cause variations in the volumes added.

- 4.5. Select the program MagMAX\_Microbiome\_Flex96\_Wastewater on the instrument.
- **4.6.** Start the run, then load the prepared sample (plate position 1) and processing plates into position when prompted by the instrument.
- **4.7.** After the protocol is complete (~35 minutes after start), immediately remove the Elution Plate from the instrument, then cover the plate or transfer the eluate to the tube or plate of choice for final storage.

# Perform SARS-CoV2 (or other viral targets) nucleic acid purification from 50 mL wastewater samples using Dynabeads<sup>™</sup> Intact Virus Enrichment beads

- Manually enrich
- 1.1. Transfer 50 mL of wastewater sample to a 50 mL falcon tube and add 750 μL of Dynabeads Intact Virus Enrichment beads.
- 1.2. Invert the tube on a HulaMixer<sup>™</sup> Sample Mixer at room temperature for 10 minutes.
- 1.3. Quickly centrifuge the tube at low speed to collect all the material on the bottom of the tube, then put the tube on the DynaMag<sup>™</sup>-50 Magnet to separate the beads.
- 1.4. Discard the supernatant, then add 2 mL of Lysis Buffer and mix the sample either by vortexing or pipetting up and down several times.
- **1.5.** Immediately put the tube on DynaMag<sup>™</sup>–50 Magnet to separate the beads.
- **1.6.** Collect the supernatant, containing the enriched and lysed virus, then add it to the appropriate wells of the designated 24 deep-well Sample Plate.

**Note:** This manual enrichment process can be used for 5 mL to 50 mL wastewater sample volumes. The bead volume can be adjusted according the sample input taken for enrichment.

- 2 Set up the instrument
- **2.1.** Ensure that the instrument is set up with the proper magnetic head and the heat block, as indicated in the following table:

Component	Туре
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.

- 2.2. Ensure that the proper program (MagMAX\_Microbiome\_Flex24\_Wastewater) has been downloaded from the product page and loaded onto the instrument.
- 3 Set up the processing plates

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	24 deep-well	Wash Buffer	2,500 µL
Wash 2 Plate	3	24 deep-well	Wash Buffer	2,500 μL
Wash 3 Plate	4	24 deep-well	80% Ethanol	2,500 µL
Wash 4 Plate	5	24 deep-well	80% Ethanol	2,500 µL
Elution Plate	6	24 deep-well	Elution Solution	100 μL <sup>[1]</sup>
Tip Comb	7	Place the 24 deep-well tip comb in a standard 24 deep-well plate		

<sup>[1]</sup> Elution volume can be reduced to 50 µL for a more concentrated sample. 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: To prevent evaporation and contamination, cover the prepared processing plates with paraffin film or MicroAmp $^{\text{TM}}$  Clear Adhesive Film until they are loaded into the instrument.

- 4 Lyse, bind, wash, then elute the total nucleic acid
- 4.1. Add 200 µL of Proteinase K to the wells of the Sample Plate containing the wastewater sample.
- 4.2. Invert the Binding Bead Mix to mix, then add 2,600 µL to each sample in the Sample Plate.

**Note:** Binding Bead Mix is viscous, so pipet slowly and mix frequently by inversion to ensure the correct volume and even distribution of beads to all the wells. DO NOT reuse pipette tips to add Binding Bead Mix to the samples, as the high viscosity will cause variations in the volumes added.

- 4.3. Select the program MagMAX\_Microbiome\_Flex24\_Wastewater on the instrument.
- **4.4.** Start the run, then load the prepared sample (plate position 1) and processing plates into position when prompted by the instrument.
- **4.5.** After the protocol is complete (~33 minutes after start), immediately remove the Elution Plate from the instrument, then cover the plate or transfer the eluate to the tube or plate of choice for final storage.

# (Option 1, 50 mL–250 mL) Perform SARS-CoV2 (or other viral targets) nucleic acid purification from wastewater samples using Nalgene<sup>™</sup> Rapid-Flow<sup>™</sup> Sterile Single Use Vacuum Filter Units beads and KingFisher<sup>™</sup> Flex

- Set up the sample plate
- 1.1. Transfer 50 mL–250 mL (depending on the input being used for extraction) of wastewater sample to multiple 50 mL falcon tubes, then centrifuge the tubes at 4,500 x *g* for 30 minutes.
- **1.2.** Collect the supernatant, then filter it through Nalgene<sup>™</sup> Rapid-Flow<sup>™</sup> Sterile Single Use Vacuum Filter Units using a vacuum pump.
- 1.3. Once filtration is finished, cut the filter unit membrane into small fragments with a razor blade.
- 1.4. Collect the membrane fragments using forceps, then add the fragments from a single filter unit to two different wells on the Bead Beating Plate, or to two different Bead Tubes.
- **1.5.** If using a Bead Beating Plate, place on high throughput homogenizer for 2 minutes. If using Bead Tubes, place on a vortexer for 10 minutes.
- 1.6. Spin down the Bead Beating Plate or Bead Tubes, then collect the supernatant from both of the wells or tubes containing the filter fragments.
- 1.7. Add 1 mL of the supernatant to the designated 24 deep-well Sample Plate.
- Set up the instrument
- **2.1.** Ensure that the instrument is set up with the proper magnetic head and the heat block, as indicated in the following table:

Component	Туре	
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.

- **2.2.** Ensure that the proper program (**MagMAX\_Microbiome\_Flex24\_Wastewater**) has been downloaded from the product page and loaded onto the instrument.
- 3 Set up the processing plates

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	24 deep-well	Wash Buffer	2,500 µL
Wash 2 Plate	3	24 deep-well	Wash Buffer	2,500 µL
Wash 3 Plate	4	24 deep-well	80% Ethanol	2,500 μL
Wash 4 Plate	5	24 deep-well	80% Ethanol	2,500 µL
Elution Plate	6	24 deep-well	Elution Solution	50 μL
Tip Comb	7	Place the 24 deep-well tip comb in a standard 24 deep-well plate		

**Note:** To prevent evaporation and contamination, cover the prepared processing plates with paraffin film or MicroAmp<sup>™</sup> Clear Adhesive Film until they are loaded into the instrument.

### 4 Elute the total nucleic acid

- 4.1. Select the program MagMAX\_Microbiome\_Flex24\_Wastewater on the instrument.
- **4.2.** Start the run, then load the prepared sample (plate position 1) and processing plates into position when prompted by the instrument.
- **4.3.** After the protocol is complete (~33 minutes after start), immediately remove the Elution Plate from the instrument, then cover the plate or transfer the eluate to the tube or plate of choice for final storage.

# (Option 2, 50 mL–250 mL) Perform SARS-CoV2 nucleic acid purification from wastewater samples using Intact Virus Precipitation Reagent and KingFisher™ Flex

- Set up the sample plate
- 1.1. Transfer 50 mL-250 mL of wastewater sample to multiple 50 mL falcon tubes, then add 10% volume of Intact Virus Precipitation Reagent (for every 33 mL of wastewater, add 17 mL of Intact Virus Precipitation Reagent, 10720D).
- 1.2. Incubate the tubes with Intact Virus Precipitation Reagent for 2 hours at 4°C.
- **1.3.** After incubation, centrifuge the tubes at 10,000 x g for 30 minutes at 4°C.
- **1.4.** Discard the supernatant, then resuspend the pellet in 2 mL of Lysis Buffer.
- 1.5. Add the 2 mL resuspension to the designated 24 deep-well Sample Plate.

**Option:** SARS-CoV2 nucleic acid isolation from sewage pellets can be performed using the *MagMAX*<sup>™</sup> *Microbiome Ultra Nucleic Acid Isolation Kit* (MAN0018071) fecal sample protocol and script.

**Note:** Sensitivity drop was observed with processing of wastewater samples using Intact Virus Precipitation Reagent, however this reagent does help with enrichment of the virus from wastewater samples.

Set up the instrument

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

Component	Туре	
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.

- **2.2.** Ensure that the proper program (**MagMAX\_Microbiome\_Flex24\_Wastewater**) has been downloaded from the product page and loaded onto the instrument.
- 3 Set up the processing plates

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	24 deep-well	Wash Buffer	2,500 µL
Wash 2 Plate	3	24 deep-well	Wash Buffer	2,500 µL
Wash 3 Plate	4	24 deep-well	80% Ethanol	2,500 µL
Wash 4 Plate	5	24 deep-well	80% Ethanol	2,500 µL
Elution Plate	6	24 deep-well	Elution Solution	50 μL
Tip Comb	7	Place the 24 deep-well tip comb in a standard 24 deep-well plate		

**Note:** To prevent evaporation and contamination, cover the prepared processing plates with paraffin film or MicroAmp<sup>™</sup> Clear Adhesive Film until they are loaded into the instrument.

### 4 Elute the total nucleic acid

- 4.1. Select the program MagMAX\_Microbiome\_Flex24\_Wastewater on the instrument.
- **4.2.** Start the run, then load the prepared sample (plate position 1) and processing plates into position when prompted by the instrument.
- **4.3.** After the protocol is complete (~33 minutes after start), immediately remove the Elution Plate from the instrument, then cover the plate or transfer the eluate to the tube or plate of choice for final storage.

### Limited product warranty

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Revision	Date	Description
A.0	22 June 2021	Baseline for this revision history.

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