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MagMAX™ Prime Viral/Pathogen NA Isolation Kit, Prefilled Plates, and Accessories

USER GUIDE

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

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B.0	29 January 2024	Prefilled plate content added.
A.0	9 November 2023	New document for the MagMAX™ Prime Viral/Pathogen NA Isolation Kit and Accessories.

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

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1 Product information

Product description

The MagMAX™ Prime Viral/Pathogen NA Isolation Kit, Prefilled Plates, and Accessories utilize magnetic bead nucleic acid purification technology intended to isolate and purify viral, bacterial, parasitic, and fungal nucleic acid from human biological samples for various downstream applications.

The product has the following features:

- Designed to isolate and purify microbial nucleic acid from a range of biological samples such as swabs in transport media, saliva, stool, urine, plasma, and serum
- Flexible workflow allowing various sample types to be batched on a single plate
- Streamlined automation ready protocols designed for basic, advanced lysis, and advanced stool workflows
- Fast automated KingFisher™ Flex, KingFisher™ Apex, and KingFisher™ Apex Dx (RUO mode) scripts to allow for 96 samples to be processed in less than 60 minutes
- Flexible protocol accommodates sample volume inputs from 200 µL to 400 µL
- Elution volume of 200 µL for stool samples and 60 µL for all other sample types
- Available user filled configuration to maximize product's flexibility and customizability
- Available pre-filled configuration to maximize user's convenience, ease of use, and efficiency
- Available lysis buffers and bead-beating accessories to extract nucleic acids from challenging organisms and raw/stabilized stool sample type

IMPORTANT! The scripts that are provided with this kit for the KingFisher™ Apex Dx instrument are for use in the RUO operating mode only. Thermo Fisher Scientific does not provide scripts for the KingFisher™ Apex Dx instrument in IVD mode.

Contents and storage

The MagMAX™ Prime Viral/Pathogen NA Isolation Kit (Cat. No. A58145) contains the following components:

Table 1 MagMAX™ Prime Viral/Pathogen NA Isolation Kit

Component	Amount	Storage
MagMAX™ Prime Viral/Pathogen Binding Solution	160 mL	15°C to 25°C
MagMAX™ Prime Viral/Pathogen Wash I Solution	300 mL	
MagMAX™ Prime Viral/Pathogen Binding Beads	6 mL	
MagMAX™ Prime Viral/Pathogen Proteinase K	3 mL	
MagMAX™ Prime Viral/Pathogen Elution Buffer	36 mL	
MagMAX™ Prime Viral/Pathogen Proteinase K dye	30 µL	

Note: Individual bottles with larger volumes can be purchased for each component. See Table 4.

Note: For workflows and workflow volumes, see “Workflow” on page 10 and “Workflow volumes” on page 11.

The MagMAX™ Prime Viral/Pathogen NA Isolation Kit, Prefilled Plates (Cat. No. A58146PF) contains sufficient reagents for 96 extractions with a 200 µL to 400 µL sample volume.

The kit contains the following components:

Table 2 MagMAX™ Prime Viral/Pathogen NA Isolation Kit, Prefilled Plates

Component	Amount	Storage
MagMAX™ Prime Viral/Pathogen Binding Solution	1 prefilled plate	15°C to 25°C
MagMAX™ Prime Viral/Pathogen Wash I Solution	1 prefilled plate	
MagMAX™ Prime Viral/Pathogen Wash II Solution	1 prefilled plate	
MagMAX™ Prime Viral/Pathogen Binding Beads	1 prefilled plate	
MagMAX™ Prime Viral/Pathogen Proteinase K	1.1 mL	
MagMAX™ Prime Viral/Pathogen Elution Buffer	1 prefilled plate	
MagMAX™ Prime Viral/Pathogen Proteinase K dye	15 µL	
96 deep-well tip comb	2 tip combs	
MicroAmp™ Clear Adhesive Film	2 films	

IMPORTANT! Store the pre-filled kit right side up as shown on the packaging.

Table 3 MagMAX™ Prime Viral/Pathogen Accessories

Component	Cat. no.	Amount	Storage
MagMAX™ Prime G+ Bacterial and Fungal Lysis Buffer	A59053	5 mL (Up to 100 preparations)	-25°C to -15°C
MagMAX™ Prime G+ Bacterial and Fungal Lysis Buffer	A58153	50 mL (Up to 1000 preparations)	
MagMAX™ Prime Stool Lysis Buffer	A58154	80 mL (Up to 100 preparations)	15°C to 25°C
MagMAX™ Prime Bead Beating Tubes	A58155	100 tubes	

MagMAX™ Prime standalone reagents are available for purchase. Information can be found below:

Table 4 MagMAX™ Prime Viral/Pathogen Standalone Reagents

Component	Cat. no.	Amount	Storage
MagMAX™ Prime Viral/Pathogen Binding Beads	A58147	20 mL	15°C to 25°C
MagMAX™ Prime Viral/Pathogen Elution Buffer	A58148	120 mL	
MagMAX™ Prime Viral/Pathogen Wash I Solution	A58149	1000 mL	
MagMAX™ Prime Viral/Pathogen Binding Solution	A58150	530 mL	
MagMAX™ Prime Viral/Pathogen Proteinase K	A58151	10 mL	
MagMAX™ Prime Viral/Pathogen Proteinase K dye	A58152	150 µL	

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

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

Item	Source
Nucleic acid purification systems	
KingFisher™ Apex with 96 Deep Well Head	5400930
KingFisher™ Apex 96 Deep Well Heating Block	24075930
KingFisher™ Apex Dx 96 Deep-Well AB Platinum Package (For RUO mode)	A59038

(continued)

Item	Source
KingFisher™ Flex Purification System with 96 Deep-Well Head	5400630
KingFisher™ Flex 96 Deep-Well Heating Block	24075430
KingFisher™ plates and tip comb[1]	
KingFisher™ 96 Deep-Well Plates (for Flex)	95040450
KingFisher™ 96 Deep-Well Plates (for Flex and Apex)	95040450B
KingFisher™ 96 tip comb for deep-well magnets (for Flex)	97002534
KingFisher™ 96 tip comb for deep-well magnets (for Flex and Apex)	97002534B
KingFisher™ 96 microplate (200 µL) (for Flex)	97002540
KingFisher™ 96 microplate (200 µL) (for Flex and Apex)	97002540B
Reagents	
Thermo Scientific™ 80% Ethanol, Molecular Biology Grade or equivalent	T08204K7
Nuclease-Free Water (not DEPC-Treated) or equivalent	4387936
Tubes, plates, and other consumables	
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Adhesive Film Applicator	4333183
Sterile conical tubes for reagent preparation	**MLS**
Sterile aerosol barrier (filtered) pipette tips	**thermofisher.com/pipettetips**
70% Isopropanol spray or wipes	**MLS**
RNase*Zap*™ RNase Decontamination Solution or equivalent	AM9780, AM9782
RNase*Zap*™ RNase Decontamination Wipes or equivalent	AM9786
Equipment	
Biosafety cabinet	**MLS**
Laboratory mixer, vortex, or equivalent	**MLS**
Tube centrifuge capable of spinning at 14000 x *g*	**MLS**
Multi-tube holder	SI-H524
Single and multichannel adjustable pipettors (1 µL to 1,000 µL)	**MLS**
Reagent reservoir	**MLS**
(Optional) Magnetic Stand-96 or equivalent	AM10027

[1] Do not use plates other than listed above. Other plates may misalign or damage the KingFisher™ instrument.

REACH statement

Thermo Fisher Scientific has evaluated the MagMAX™ Prime Viral/Pathogen NA Isolation Kit and confirms that it does not contain substances of very high concern (SVHCs) or substances on the Annex XIV or XVII list, as defined by *Regulation (EC) No 1907/2006—Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)*.

Warnings and precautions

The MagMAX™ Prime Viral/Pathogen NA Isolation Kit workflow should be performed by qualified and trained staff to avoid the risk of erroneous results. Use separate areas for the preparation of samples and controls to prevent cross-contamination.

- Samples should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Follow necessary precautions when handling samples. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free from DNases and RNases.
- Do not eat, drink, smoke, or apply cosmetic products in the work areas.
- Do not use the kit after the expiration date.
- Reagents contain guanidine isothiocyanate. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially hazardous chemicals.
- Safety Data Sheets are available upon request.
- Reagents must be stored and handled as specified. See “Contents and storage” on page 6.

IMPORTANT! Store the pre-filled kit right side up as shown on the packaging.

General laboratory recommendations

- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of samples can affect extraction quality.
- Follow the sample inactivation guidelines and requirements established by your laboratory and local regulatory bodies. If your laboratory inactivates samples, do so before you begin the workflow.
- Implement standard operating procedures in your laboratory to prevent contamination, such as the following:
  - Frequent glove changes
  - Frequent decontamination of surfaces, equipment, and pipettes with 10% bleach (1% V/V sodium hypochlorite) or decontamination solution, followed by 70% ethanol

Note: The MagMAX™ Prime G+ Bacterial and Fungal Lysis Buffer is purified from *Cellulosimicrobium cellulans* expressing a proprietary version of the enzyme. Trace amounts of host nucleic acid may be present in the final formulation. When performing pre-amplification, there is a possibility of slight background amplification if the assays are not highly specific to the target pathogen.

Workflow

Table 5 User Filled Reagent Workflow

Workflow	Pre-processing accessories	Sample types	Pathogens	Script[1]
Basic: 200 µL fixed volume input (See page 13)	N/A	Swabs in transport media and saliva	• Virus • Gram (-) bacteria • *Staphylococcus aureus*	KingFisher™ Flex: **Prime_FLX** KingFisher™ Apex: **Prime_APX**
Basic: 200 µL to 400 µL scalable volume (See page 13)		Swabs in transport media, saliva, urine, plasma, and serum		
Advanced lysis: 200 µL to 400 µL scalable volume (See page 20)	MagMAX™ Prime G+ Bacterial and Fungal Lysis Buffer	Swabs in transport media, saliva, urine, plasma, and serum	• Virus • Gram (-) bacteria • Gram (+) bacteria • Fungi	KingFisher™ Flex: **Prime_GPB_Fungi_FLX** KingFisher™ Apex: **Prime_GPB_Fungi_APX**
Advanced stool (See page 26)	MagMAX™ Prime Stool Lysis Buffer MagMAX™ Prime Bead Beating Tubes	Fecal swab in transport media, raw stool, and stool in media/stabilization solution	• Virus • Gram (-) bacteria • Gram (+) bacteria • Parasites • Fungi	KingFisher™ Flex: **Prime_GI_FLX** KingFisher™ Apex: **Prime_GI_APX**

[1] The script file extension is .bdz for the KingFisher™ Flex and .kfx for the KingFisher™ Apex GPLE instrument. The .kfx script files can be used with KingFisher™ Apex Dx instrument in RUO mode. Thermo Scientific™ does not provide script used with KingFisher™ Apex Dx instrument IVD mode for this kit

Table 6 Prefilled Reagent Workflow

Workflow	Pre-processing accessories	Sample types	Pathogens	Script
Basic: 200 µL to 400 µL scalable volume (See page 18)	N/A	Swabs in transport media, saliva, urine, plasma, and serum	• Virus • Gram (-) bacteria • *S. aureus*	KingFisher™ Flex: **Prime_Prefill_FLX** KingFisher™ Apex: **Prime_Prefill_APX**
Advanced lysis: 200 µL to 400 µL scalable volume (See page 24)	MagMAX™ Prime G+ Bacterial and Fungal Lysis Buffer	Swabs in transport media, saliva, urine, plasma, and serum	• Virus • Gram (-) bacteria • Gram (+) bacteria • Parasites • Fungi	KingFisher™ Flex: **Prime_GPB_Fungi_Prefill_FLX** KingFisher™ Apex: **Prime_GPB_Fungi_Prefill_APX**

Workflow volumes

Workflow	Sample input	Sample pre-processing	Bead binding solution + beads (bead binding mixture)	Wash I solution	80% ethanol solution	Elution buffer	Preparation
Basic	200 µL fixed volume	None	265 µL + 10 µL	500 µL	500 µL	60 µL	up to 600 extractions
	200 µL to 400 µL scalable volume	None	500 µL + 15 µL	750 µL	750 µL	60 µL	up to 300 extractions
Advanced lysis	200 µL to 400 µL scalable volume	50 µL of MagMAX™ Prime G+ Bacterial and Fungal Lysis Buffer	500 µL + 15 µL	750 µL	750 µL	60 µL	up to 300 extractions
Advanced stool	400 µL fixed volume	800 µL of MagMAX™ Prime Stool Lysis Buffer 1 MagMAX™ Prime Bead Beating Tube	500 µL + 15 µL	950 µL	950 µL	200 µL	up to 150 extractions

Before you begin

IMPORTANT! Do not mix Binding Bead Mix with bleach. For more information, see the SDS.

Note: The Wash Solution and Binding Solution may develop inert white or brown particulates that float in solution. The Wash Solution and Binding Solution can also turn yellow in color if exposed to high intensity light or high temperature. This is not a cause for concern and does not affect performance.

- Samples must be collected and stored according to laboratory guidelines.
- Ensure that you read and understand the information provided in this guide before you begin the extraction procedure.
- Avoid generating bubbles with binding and wash reagents. Mix reagents by slow inversion or slowly pipetting up and down.
- Determine the number of required extractions to be processed, plus one negative control recomemnded per plate.
- Ensure that 80% ethanol is available for use.
- For stabilized saliva collection devices, follow manufacturer's recommendation.

2 Extract nucleic acid using the basic workflow via user filled method

Basic workflow

Table 7 200 µL fixed volume workflow

Pre-processing accessories	Sample types	Pathogens	Script
N/A	Swabs in transport media and saliva	• Virus • Gram (-) bacteria • *S. Aureus*	KingFisher™ Flex: **Prime_FLX** KingFisher™ Apex: **Prime_APX**

Table 8 200 µL to 400 µL scalable volume workflow

Pre-processing accessories	Sample types	Pathogens	Script
N/A	Swabs in transport media, saliva, urine, plasma, and serum	• Virus • Gram (-) bacteria • *S. Aureus*	KingFisher™ Flex: **Prime_FLX** KingFisher™ Apex: **Prime_APX**

Before you begin

- Label the short side (next to column 12) of each KingFisher™ 96 Deep-Well Plates:

Label	Number of plates
Sample Plate	1
Wash I Plate	1
Wash II Plate	1
Elution Plate	1

- Label the short side (next to column 12) of the KingFisher™ 96 microplate (200 µL) or KingFisher™ 96 Deep-Well Plates:

Label	Number of plates
Tip Comb Plate	1

Set up the instrument

1. Ensure that the deep-well heating block is installed on the KingFisher™ Apex with 96 Deep Well Head or KingFisher™ Flex Purification System with 96 Deep-Well Head.

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

2. Ensure that the correct script (**Prime_APX** script for the KingFisher™ Apex instrument or **Prime_FLX** script for the KingFisher™ Flex instrument) has been downloaded from the MagMAX™ Prime Viral/Pathogen NA Isolation Kit product page and loaded onto the instrument.

Prepare processing plates

Note:

- Do not add reagents to wells that will not contain sample or control.
- For consistent liquid dispensing across the plate, pre-wet the pipette tip two times. Dispense the liquid by pressing the pipette plunger only to the point of first resistance. If using a multichannel pipette, the same set of pipette tips can be reused to fill all 96 wells (for a given reagent) of a KingFisher™ 96 Deep-Well Plate. Use this technique for filling all reagents.

1. Prepare the processing plates according to the following table. Cover the plates with a temporary seal, then store at room temperature while the Sample Plate is prepared.

Plate ID	Plate type	Reagent	Volume per well	
			200 µL fixed volume workflow	200 µL to 400 µL scalable volume workflow
Wash I Plate	KingFisher™ 96 Deep-Well Plate	Wash Solution	500 µL	750 µL
Wash II Plate		80% Ethanol solution	500 µL	750 µL
Elution Plate		Elution Buffer	60 µL	60 µL
Tip Comb Plate	Place a KingFisher™ 96 tip comb for deep-well magnets in a KingFisher™ 96 microplate (200 µL)			

2. *(Optional)* Add 10 µL of Proteinase K dye to 1 mL of Proteinase K in a separate 1.5 mL tube then mix by vortexing gently.

Note: Proteinase K dye in Proteinase K serves a visual purpose only. It allows easy tracking of the samples which have received Proteinase K.

Prepare Binding Bead Mix

Prepare the required amount of Binding Bead Mix before each use.

1. Vortex the Binding Beads, ensuring that the bead mixture is homogeneous.
2. For the number of required extractions, prepare the Binding Bead Mix according to the following table.

Component	Volume per well[1]	
	200 µL fixed volume workflow	200 µL to 400 µL scalable volume workflow
Binding Solution	265 µL	500 µL
Binding Beads	10 µL	15 µL
Total volume per well	275 µL	515 µL

[1] Include 10% overage when making the Binding Bead Mix for use with multiple extractions. These volumes do not include for the recommended overage.

Note: The Binding Bead Mix is stable for 4 hours at room temperature.

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

Note: Avoid generating bubbles when mixing. Bubbles can occur during vigorous pipetting up and down, shaking, or a combination of the two.

Prepare the sample plate

1. Invert the Binding Bead Mix 5 times gently to mix, then add Binding Bead Mix to each sample well and to the Negative Control well in the Sample Plate (KingFisher™ 96 Deep-Well Plate). See table below.

Component	Volume	
	200 µL fixed volume workflow	200 µL to 400 µL scalable volume workflow
Binding Bead Mix	275 µL	515 µL

Note:

- Do not add Binding Bead Mix or other reagents to wells that have no sample or control.
- To ensure even distribution of beads to all samples or wells, mix the Binding Bead Mix frequently during pipetting. The Binding Bead Mix is viscous, so pipet slowly to ensure that the correct amount is added.

IMPORTANT! Add the components only to the top layer of the solution in each well. Do not push the pipette tip into the binding mix layer.

2. Add the desired volume of sample (200 µL to 400 µL) to each sample well.

Note: You may observe a color change when sample is added to the Binding Bead Mix. This does not affect performance of the kit.

3. Add the appropriate volume of nuclease-free water to the control well.

Component	Volume	
	200 µL fixed volume workflow	200 µL to 400 µL scalable volume workflow
Nuclease-free water	200 µL	200 µL to 400 µL[1]

[1] The volume of nuclease-free water must equal the sample volume.

4. Add the following components to each sample and control well.

Component	Volume	
	200 µL fixed volume workflow	200 µL to 400 µL scalable volume workflow
Proteinase K or Proteinase K dye mixture	5 µL	10 µL

Note: Proteinase K dye in Proteinase K serves visual purposes only. It allows easy tracking of the samples which have receive Proteinase K.

Process the samples

1. Select the **Prime_APX** script for the KingFisher™ Apex instrument or **Prime_FLX** script for the KingFisher™ Flex instrument.

Note: Please check the MagMAX™ Prime Viral/Pathogen NA Isolation Kit product page for the most current version of the script.

2. Start the run, then load the prepared plates into position as prompted by the instrument.

   Note: Once prepared, the plates must be loaded on the instrument within 30 minutes.

   IMPORTANT! When loading plates, ensure that the A1 position of plate aligns with the A1 position indicated on the plate stations of the turntable in the instrument.

3. After the program runs for approximately 30 minutes, remove the Elution Plate within 10 minutes, and cover the plate with a clear adhesive film. Promptly place the Elution Plate on ice. The extraction is complete.

   Note: The purified nucleic acid is ready for immediate use. Alternatively, store the plate at –20°C for long term storage.

   The samples are eluted in 60 µL of Elution Buffer.

3 Extract nucleic acid using the basic workflow via the prefilled plate method

Basic workflow

Pre-processing accessories	Sample types	Pathogens	Script
N/A	Swabs in transport media, saliva, urine, plasma, and serum	• Virus • Gram (-) bacteria • *S. aureus*	KingFisher™ Flex: **Prime_Prefill_FLX** KingFisher™ Apex: **Prime_Prefill_APX**

Before you begin

- Remove the Sample Plate and the vials of Proteinase K and Proteinase K dye from the product box.
- *(Optional)* Add 10 µl of Proteinase K dye to the vial of Proteinase K and mix by vortexing.
- Store the pre-filled kit right side up as shown on the packaging.

Process the samples

1. Remove the sealing film of the Sample Plate, then add 200 µL to 400 µL of sample to each well.
2. Add 10 µL of Proteinase K to each sample well.
3. When ready to begin the run, click **Start**. Peel off the sealing film of each pre-filled deep-well plate prior to loading onto the instrument.
4. Select the **Prime_Prefill_APX** script for the KingFisher™ Apex instrument or the **Prime_Prefill_FLX** script for the KingFisher™ Flex instrument.
5. Place a 96 deep-well tip comb into the Binding Bead plate.

Note: Do not place the tip comb into the Sample Plate.

6. Load each plate into the corresponding deck position following the instrument prompts.

Deck position	Prefilled plate	Contents	Prefilled volume
1	MagMAX™ Prime Viral/Pathogen Binding Beads (bead plate)	Magnetic beads	400 µL
2	MagMAX™ Prime Viral/Pathogen Binding Solution (sample plate)	Binding solution + proteinase K + sample	Proteinase K: 10 µL Sample: 200 µL–400 µL Binding solution: 500 µL
3	MagMAX™ Prime Viral/Pathogen Wash I Solution (wash I)	Wash I solution	Wash I solution: 750 µL
4	MagMAX™ Prime Viral/Pathogen Wash II Solution (wash II)	Wash II solution (80% ethanol)	Wash II solution: 750 µL
5	MagMAX™ Prime Viral/Pathogen Elution Buffer (elution plate)	Elution buffer	Elution Buffer: 60 µL

Note: Place the prefilled plate as indicated and check the deck position number. Deck positions are also denoted on labels on the short and long ends of the plate. Ensure that plates are seated firmly and correctly on the instrument before starting a run.

IMPORTANT! When loading plates, ensure that the A1 position of plate aligns with the A1 position indicated on the plate stations of the turntable in the instrument.

7. After the program runs for approximately 30 minutes, remove the Elution Plate within 10 minutes, and cover the plate with a clear adhesive film. Promptly place the Elution Plate on ice. The extraction is complete.

Note: The purified nucleic acid is ready for immediate use. Alternatively, store the plate at –20°C for long term storage.

4 Extract nucleic acid from difficult to lyse pathogens using the advanced lysis workflow via user filled method

Advanced lysis workflow

Pre-processing accessories	Sample types	Pathogens	Script
MagMAX™ Prime G+ Bacterial and Fungal Lysis Buffer	Swabs in transport media, saliva, urine, plasma and serum	• Virus • Gram (-) bacteria • Gram (+) bacteria • Fungi	KingFisher™ Flex: **Prime_GPB_Fungi_FLX** KingFisher™ Apex: **Prime_GPB_Fungi_APX**

Before you begin

- Label the short side (next to column 12) of each KingFisher™ 96 Deep-Well Plates:

Label	Number of plates
Sample Plate	1
Wash I Plate	1
Wash II Plate	1
Elution Plate	1

- Label the short side (next to column 12) of the KingFisher™ 96 microplate (200 µL) or KingFisher™ 96 Deep-Well Plates:

Label	Number of plates
Tip Comb Plate	1

Set up the instrument

1. Ensure that the deep-well heating block is installed on the KingFisher™ Apex with 96 Deep Well Head or KingFisher™ Flex Purification System with 96 Deep-Well Head.

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

2. Ensure that the correct script (**Prime_GPB_Fungi_APX** script for the KingFisher™ Apex instrument or the **Prime_GPB_Fungi_FLX** script for the KingFisher™ Flex instrument) has been downloaded from the MagMAX™ Prime Viral/Pathogen NA Isolation Kit product page and loaded onto the instrument.

Prepare processing plates

Note:

- Do not add reagents to wells that will not contain sample or control.
- For consistent liquid dispensing across the plate, pre-wet the pipette tip two times. Dispense the liquid by pressing the pipette plunger only to the point of first resistance. If using a multichannel pipette, same set of pipette tips can be reused to fill all 96 wells of a KingFisher™ 96 Deep-Well Plate. Use this technique for filling all reagents.

1. Prepare the processing plates according to the following table. Cover the plates with a temporary seal, then store at room temperature while you set up the Sample Plate.

Plate ID	Plate type	Reagent	Volume per well
			200 µL to 400 µL sample volume
Wash I Plate	KingFisher™ 96 Deep-Well Plate	Wash Solution	750 µL
Wash II Plate		80% Ethanol solution	750 µL
Elution Plate		Elution Buffer	60 µL
Tip Comb Plate	Place a KingFisher™ 96 tip comb for deep-well magnets in a KingFisher™ 96 microplate (200 µL)		

2. *(Optional)* Add 10 µL of Proteinase K dye to 1 mL of Proteinase K in a separate 1.5 mL tube then mix by vortexing gently.

   Note: Proteinase K dye in Proteinase K serves visual purpose only. It allows easy tracking of the samples which have received Proteinase K.

Digest sample using MagMAX™ Prime G+ Bacterial and Fungal Lysis Buffer

1. Add 50 µl MagMAX™ Prime G+ Bacterial and Fungal Lysis Buffer to each well in a 96 deep-well plate.
2. Add 200 µL to 400 µL of sample.
3. Select the **Prime_GPB_Fungi_APX** script for the KingFisher™ Apex instrument or the **Prime_GPB_Fungi_FLX** script for the KingFisher™ Flex instrument.
4. Click **start**, then load the prepared plates into position when prompted by the instrument.

IMPORTANT! When loading plates, ensure that the A1 position of plate aligns with the A1 position indicated on the plate stations of the turntable in the instrument.

Note: The run will pause after approximately 20 minutes for the addition of Proteinase K and binding bead mix in the sample plate.

Prepare binding bead mix

Prepare the required amount of Binding Bead Mix before each use.

1. Vortex the Binding Beads, ensuring that the bead mixture is homogeneous.
2. For the number of required extractions, prepare the Binding Bead Mix according to the following table.

Component	Volume per well
	200 µL to 400 µL sample volume
Binding Solution	500 µL
Binding Beads	15 µL
Total volume per well	515 µL

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

Note: Avoid generating bubbles when mixing. Bubbles can occur during vigorous pipetting up and down, shaking, or a combination of the two.

Process the samples

1. When prompted (approximately 20 minutes after start of protocol), remove Sample Plate from instrument.
2. Add 10 µL of Proteinase K or Proteinase K dye mixture to each sample in the Sample Plate.

3. Invert Binding Bead Mix gently to mix, then add 515 µL to each sample in the Sample Plate.

   Note: Remix the Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. The mixture containing the Binding Beads is viscous. Therefore, pipette slowly to ensure that the correct amount is added.

4. Load the Sample Plate back onto the instrument, then click **Start**.

5. After the program runs for approximately 30 minutes, remove the Elution Plate within 10 minutes, and cover the plate with a clear adhesive film. Promptly place the Elution Plate on ice. The extraction is complete.

   Note: The purified nucleic acid is ready for immediate use. Alternatively, store the plate at –20°C for long term storage.

5 Extract nucleic acid from difficult to lyse pathogens using the advanced lysis workflow via the prefilled plate method

Advanced lysis workflow

Pre-processing accessories	Sample types	Pathogens	Script
MagMAX™ Prime G+ Bacterial and Fungal Lysis Buffer	Swabs in transport media, saliva, urine, plasma, and serum	• Virus • Gram (-) bacteria • Gram (+) bacteria • Parasites • Fungi	KingFisher™ Flex: **Prime_GPB_Fungi_Prefill_FLX** KingFisher™ Apex: **Prime_GPB_Fungi_Prefill_APX**

Before you begin

- Remove the Sample Plate, prefilled plates, and the vials of Proteinase K and Proteinase K Dye from the product box.
- *(Optional)* Add 10 µl of Proteinase K dye to the vial of Proteinase K and mix by vortexing gently.
- Store the pre-filled kit right side up as shown on the packaging.

Digest and process samples using MagMAX™ Prime G+ Bacterial and Fungal Lysis Buffer

1. Add 50 µl MagMAX™ Prime G+ Bacterial and Fungal Lysis Buffer to each well in a 96 deep-well plate.

   IMPORTANT! For the sample digestion step, users need an additional empty 96 deep-well Lysis plate which is not supplied with the kit.

2. Add 200 µL to 400 µL of sample to each sample well.

3. Select the **Prime_GPB_Fungi_Prefill_APX** script for the KingFisher™ Apex instrument or the **Prime_GPB_Fungi_Prefill_FLX** script for the KingFisher™ Flex instrument.

4. Place a 96 deep-well tip comb into the Binding Bead plate.

Note: Do not place tip comb into any other plate than the Binding Bead plate.

5. Load each plate into the corresponding deck position following the instrument prompts.

Deck position	Prefilled plate	Contents	Prefilled volume
1	MagMAX™ Prime Viral/Pathogen Binding Beads (bead plate)	Magnetic beads	400 µL
2	MagMAX™ Prime Viral/Pathogen Binding Solution (sample plate)	Binding solution + proteinase K + sample	Proteinase K: 10 µL Sample: 200 µL–400 µL Binding solution: 500 µL
3	MagMAX™ Prime Viral/Pathogen Wash I Solution (wash I)	Wash I solution	Wash I solution: 750 µL
4	MagMAX™ Prime Viral/Pathogen Wash II Solution (wash II)	Wash II solution (80% ethanol)	Wash II solution: 750 µL
5	MagMAX™ Prime Viral/Pathogen Elution Buffer (elution plate)	Elution buffer	Elution Buffer: 60 µL

6. When prompted, remove Lysis plate from instrument.

Note: The run will pause after approximately 20 minutes for the transfer of digested sample to the plate containing binding solution.

7. Transfer digested sample to the plate containing binding solution.

8. Add 10 µL of Proteinase K to each sample well or control well.

9. Follow the directions on the instrument screen. Return Binding Solution plate with sample to instrument to complete the extraction process.

10. After the program runs for approximately 30 minutes, remove the Elution Plate within 10 minutes, and cover the plate with a clear adhesive film. Promptly place the Elution Plate on ice. The extraction is complete.

Note: The purified nucleic acid is ready for immediate use. Alternatively, store the plate at –20°C for long term storage.

6 Extract nucleic acid from raw or stabilized stool sample using the advanced workflow via the user filled method

Advanced stool workflow

Pre-processing accessories	Sample types	Pathogens	Script
MagMAX™ Prime Stool Lysis Buffer MagMAX™ Prime Bead Beating Tubes	Fecal swab in transport media, raw stool, and stool in media/stabilization solution	• Virus • Gram (-) bacteria • Gram (+) bacteria • Parasites • Fungi	KingFisher™ Flex: **Prime_GI_FLX** KingFisher™ Apex: **Prime_GI_APX**

Before you begin

- Label the short side (next to column 12) of each KingFisher™ 96 Deep-Well Plates:

Label	Number of plates
Sample Plate	1
Wash I Plate	1
Wash II Plate	1
Elution Plate	1

- Label the short side (next to column 12) of the KingFisher™ 96 microplate (200 µL) or KingFisher™ 96 Deep-Well Plates:

Label	Number of plates
Tip Comb Plate	1

Set up the instrument

1. Ensure that the deep-well heating block is installed on the KingFisher™ Apex with 96 Deep Well Head or KingFisher™ Flex Purification System with 96 Deep-Well Head.

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

2. Ensure that the correct script (**Prime_GI_APX** script for the KingFisher™ Apex instrument or **Prime_GI_FLX** script for the KingFisher™ Flex instrument) has been downloaded from the MagMAX™ Prime Viral/Pathogen NA Isolation Kit product page and loaded onto the instrument.

Prepare processing plates

Note:

- Do not add reagents to wells that will not contain sample or control.
- For consistent liquid dispensing across the plate, pre-wet the pipette tip two times. Dispense the liquid by pressing the pipette plunger only to the point of first resistance. If using a multichannel pipette, same set of pipette tips can be reused to fill all 96 wells of a KingFisher™ 96 Deep-Well Plate. Use this technique for filling all reagents.

1. Prepare the processing plates according to the following table. Cover the plates with a temporary seal, then store at room temperature while you set up the Sample Plate.

Plate ID	Plate type	Reagent	Volume per well
			400 µL sample volume
Wash I Plate	KingFisher™ 96 Deep-Well Plate	Wash Solution	950 µL
Wash II Plate		80% Ethanol solution	950 µL
Elution Plate		Elution Buffer	200 µL
Tip Comb Plate	Place a KingFisher™ 96 tip comb for deep-well magnets in a KingFisher™ 96 microplate (200 µL)		

2. *(Optional)* Add 10 µl of Proteinase K dye to 1 mL of Proteinase K in a separate 1.5 mL tube then mix by vortexing.

   Note: Proteinase K dye in Proteinase K serves visual purpose only. It allows easy tracking of the samples which have received Proteinase K.

Prepare binding bead mix

Prepare the required amount of Binding Bead Mix before each use.

1. Vortex the Binding Beads, ensuring that the bead mixture is homogeneous.
2. For the number of required extractions, prepare the Binding Bead Mix according to the following table.

Component	Volume per well[1]
	400 µL sample volume
Binding Solution	500 µL
Binding Beads	15 µL
Total volume per well	515 µL

[1] Include 10% overage when making the Binding Bead Mix for use with multiple extractions. The volumes listed in the table do not include the overage.

Note: The Binding Bead Mix is stable for 4 hours at room temperature.

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

Note: Avoid generating bubbles when mixing. Bubbles can occur during vigorous pipetting up and down, shaking, or a combination of the two.

Pre-process stool sample using bead beating tubes

Set up the Vortex with the vortex adaptor.

1. Add 800 µL of MagMAX™ Prime Stool Lysis Buffer to the bead beating tubes.
2. Prepare fecal samples according to the following table.

For	Description
Fresh or frozen fecal sample	Weigh out 100 mg, then place in prepared bead tube
Fecal sample in storage solution (e.g. Cary Blair Media)	Collect 200 µL, then place in prepared bead tube
Fecal swab in storage solution	Collect 200 µL, then place in prepared bead tube

3. Add 20 µL Proteinase K or Proteinase K dye mixture to each tube after adding sample.
4. Cap, then vortex the bead tube upside down for 10 seconds to mix the sample with the buffer.

5. Set the vortex speed to 2,500 rpm, then place the tubes onto the adapter. Vortex samples at 2,500 rpm for 10 minutes.

   Note: We recommend the Scientific Industries SI-A236 Digital Vortex-Genie 2 with SI-H524 tube adapter and vortex for maximum nucleic acid yield. If an alternative instrument or adapter is used, ensure that the instrument can meet the speed listed in the protocol.

6. Remove tubes from vortex, then centrifuge at 14,000 × *g* for 2 minutes.

7. Transfer 400 µL of the supernatant from bead tubes to the appropriate wells of a deep-well Sample Plate.

   Note: Aspirate 400 µL supernatant carefully such that the pellet/debris is not disturbed. Transfer of the debris to sample plate might affect the performance.

8. Invert Binding Bead Mix to mix, then add 515 µL to each sample in the Sample Plate.

   Note: Remix the Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. The mixture containing the Binding Beads is viscous. Therefore, pipette slowly to ensure that the correct amount is added.

9. Select the **Prime_GI_APX** script for the KingFisher™ Apex instrument or **Prime_GI_FLX** script for the KingFisher™ Flex instrument.

10. Click **Start**, then load the prepared sample and processing plates into position when prompted by the instrument.

    IMPORTANT! When loading plates, ensure that the A1 position of plate aligns with the A1 position indicated on the plate stations of the turntable in the instrument.

11. After the program runs for approximately 45 minutes, remove the Elution Plate within 10 minutes, and cover the plate with a clear adhesive film. Promptly place the Elution Plate on ice. The extraction is complete.

    Note: The purified nucleic acid is ready for immediate use. Alternatively, store the plate at –20°C for long term storage.

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Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311
- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf

WARNING! Potential Biohazard. If you use the kit with the automated nucleic acid extraction workflow, the surface of the may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

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Documentation and support

Customer and technical support

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- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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**.