applied biosystems

MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit

INSTRUCTIONS FOR USE

Catalog Number A48383

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Revision D.0

CE IVD

For In Vitro Diagnostic Use.

ThermoFisher SCIENTIFIC

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

Revision history: Pub. No. MAN0019746

Revision	Date	Description
D.0	4 August 2022	• A REACH Regulation statement was added ("REACH statement" on page 6). • A reference to *In Vitro Diagnostic Regulation (EU) 2017/746* was added ("Warnings and precautions" on page 8). • The required materials list was rearranged for clarity ("Required materials not supplied" on page 6). • A new section about in-use reagent stability was added ("In-use reagent stability" on page 9). • Notes were added to the extraction procedures to avoid bubbles during mixing steps and avoid adding reagents to wells with no sample or control. • Notes were added to the extraction procedures about a color change in the Binding Bead Mix and particulates in the Wash Solution and Binding Solution, which do not affect performance. • A new chapter on performance characteristics was added (Chapter 4, "Performance characteristics"). • Minor updates were made throughout for consistency of style and terminology.
C.0	28 July 2021	• Updated the intended use to include saliva. • Added references to the *MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit Supplemental Instructions* (Pub. No. MAN0025302). • Reorganized "Before you begin" and stability information for clarity.
B.0	27 May 2021	• Added Cat. No. A5400630 for the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head. • Added stability information. • *(400-µL sample input volume only)* For the Proteinase K digestion procedure, changed the incubation time to 10 minutes.
A.0	20 November 2020	New document.

The customer is responsible for compliance with regulatory requirements that pertain to their procedures and uses of the instrument.

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

Contents

1 Product information

Intended use

The MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit is a nucleic acid purification kit based on magnetic bead technology. The kit is intended for the isolation and purification of viral nucleic acids from human nasopharyngeal swabs and viral RNA from saliva specimens, and is suitable for use in molecular detection by qPCR.

The kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of magnetic bead purification, either manual or automated, and *in vitro* diagnostic procedures.

Product description

The MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit (Cat. No. A48383) is specifically designed to recover RNA and DNA from viral particles contained in transport medium. The kit uses MagMAX™ magnetic-bead technology to provide reproducible recovery of high-quality nucleic acid.

This product is intended for *in-vitro* diagnostic use and includes the following features:

- Automated workflow using the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head allows for 96 nasopharyngeal swab or saliva specimens to be processed in <30 minutes
- Protocol options support an automated workflow or a manual workflow
- Flexible protocol accommodates specimen volumes from 200–400 µL of transport medium
- No need for carrier RNA
- Elution volume of 50 µL

Note: This guide includes protocols for nasopharyngeal swab specimens. For saliva specimens, see *MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit Supplemental Instructions* (Pub. No. MAN0025302).

Contents and storage

The MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit contains sufficient reagents for 1,000 extractions with a 400-µL specimen volume or 2,000 extractions with a 200-µL specimen volume.

Component	Amount	Storage
Binding Solution	550 mL	15°C to 25°C
Wash Solution	1,000 mL	
Binding Beads	20 mL	
Proteinase K	10 mL	
Elution Buffer	100 mL	

REACH statement

Thermo Fisher Scientific has evaluated the MagMAX™ Viral/Pathogen II Nucleic Acid 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)*.

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
Automated nucleic acid extraction system and materials	
KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head	5400630, A5400630
KingFisher™ Flex 96 Deep-Well Heating Block	24075430
KingFisher™ 96 Deep-Well Plate	95040450, A48305, A48424, 95040455

(continued)

Item	Source
Use one of the following plates for the Tip Comb Plate. Do not use plates that are not listed here when performing a KingFisher™ automated protocol because other plates may misalign or damage the KingFisher™ instrument: • KingFisher™ 96 KF microplate • Tip Comb Presenting Plate for KF 96 • Nunc™ MicroWell™ 96-Well Microplate, Flat Bottom • Nunc™ MicroWell™ 96-Well Microplate, barcoded • Abgene™ 96-Well Polypropylene Storage Microplate • Abgene™ 96-Well 1.2-mL Polypropylene Deepwell Storage Plate • Nunc™ F96 MicroWell™ Black Polystyrene Plate • Nunc™ F96 MicroWell™ White Polystyrene Plate • KingFisher™ 96 Deep-Well Plate	 • 97002540 • 267600 • 167008 • 269787 • AB0796 • AB1127 • 137101 • 136101 • 95040450, A48305, A48424, 95040455
KingFisher™ Deep Well 96 Tip Comb	97002534, A48438
Manual nucleic acid extraction system and materials	
Magnetic Stand-96	AM10027, AM10050
Compact Digital Microplate Shaker	88882005
Incubator capable of reaching 65°C with slatted shelves	MLS
KingFisher™ 96 Deep-Well Plate	95040450, A48305, A48424, 95040455
Use one of the following plates for the Elution Plate (manual extraction): • KingFisher™ 96 KF microplate • MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL • MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL • MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 0.2 mL • MicroAmp™ Optical 96-Well Reaction Plate, 0.2 mL	 • 97002540 • 4346906, 4366932 • 4346907 • 4306737, 4326659 • N8010560, 4316813
Equipment	
Laboratory mixer, vortex or equivalent	MLS
Single and multichannel adjustable pipettors (1.00 µL to 1,000.0 µL)	MLS
Cold block or ice	MLS

(continued)

Item	Source
Reagents	
Fisher BioReagents™ Ethanol, Absolute, Molecular Biology Grade[1], or equivalent	BP2818100, BP2818500, BP28184
Nuclease-Free Water (not DEPC-Treated), or equivalent	4387936
(*Optional*) Extraction control, if required for your assay	See the assay guide for more information
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**

[1] Available at **fisherscientific.com**.

Warnings and precautions

The MagMAX™ Viral/Pathogen II Nucleic Acid 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 patient specimens and controls to prevent false positive results. Specimens and reagents must be handled under a laminar airflow hood or biological safety cabinet.

- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the relevant regulatory authority in which the user and/or the patient is established.
- Specimens should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Follow necessary precautions when handling specimens. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious specimens.
- 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.
- Modifications to reagents, protocols, or instrumentation are not permitted, and are in violation of *In Vitro Diagnostic Regulation (EU) 2017/746*.
- Do not use the kit after the expiration date.
- Dispose of waste in compliance with the local regulations.
- Safety Data Sheets are available upon request.
- Reagents must be stored and handled as specified in “Contents and storage” on page 6.

General laboratory recommendations

- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may 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
- The protocol should be performed by qualified and trained clinical laboratory personnel.

In-use reagent stability

Reagent or plate	Stability information
Open reagent bottles or reagents poured into reagent reservoirs	Stable for ≤2 hours at room temperature.
Prepared processing plates with a temporary seal	Stable for ≤1 hour at room temperature while you set up the Sample Plate.
Binding Bead Mix	Stable for ≤8 hours at room temperature.
Proteinase K and extraction control mix	Stable for ≤8 hours on ice.
Prepared Sample Plate with samples added	Stable for ≤30 minutes before starting a run.

2 Extract nucleic acid from swab-based specimens (automated method)

Automated nucleic acid extraction is performed with the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head using a specimen volume of 200 µL or 400 µL. See your assay documentation for specific specimen volume recommendations.

Before you begin—Automated method

IMPORTANT! The Binding Bead Mix is not compatible 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. This is not a cause for concern and does not negatively affect performance.

- Patient 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.
- Review your assay documentation to determine if an extraction control is recommended to verify the efficacy of the nucleic acid preparation. Follow the extraction control guidelines provided in the assay documentation.
- 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 per plate.
- Prepare fresh 80% ethanol solution using Fisher BioReagents™ Ethanol, Absolute, Molecular Biology Grade and nuclease-free water. Prepare sufficient volume for the required number of extractions plus 10% overage.

Specimen volume	Volume of 80% ethanol solution per extraction
200 µL	500 µL
400 µL	1,000 µL

- Note the following for all kit components and 80% ethanol solution: Open reagent bottles, or reagents poured in reagent reservoirs, have been shown to be stable for ≤2 hours at room temperature.
- Label the short side (next to column 12) of each KingFisher™ 96 Deep-Well Plate (4):

Label	Number of plates
Sample Plate	1
Wash Plate 1	1
Wash Plate 2	1
Elution Plate	1

- Label the short side (next to column 12) of the KingFisher™ 96 KF microplate (1):

Label	Number of plates
Tip Comb Plate	1

Note: The following items can be used to hold the tip comb instead of the KingFisher™ 96 KF microplate:

- Tip Comb Presenting Plate for KF 96
- Nunc™ MicroWell™ 96-Well Microplate, Flat Bottom
- Nunc™ MicroWell™ 96-Well Microplate, barcoded
- Abgene™ 96-Well Polypropylene Storage Microplate
- Abgene™ 96-Well 1.2-mL Polypropylene Deepwell Storage Plate
- Nunc™ F96 MicroWell™ Black Polystyrene Plate
- Nunc™ F96 MicroWell™ White Polystyrene Plate
- KingFisher™ 96 Deep-Well Plate

- Mark the Negative Control well on the Sample Plate and the Elution Plate.

Extract nucleic acid—Automated method (200-µL specimen volume)

Set up the instrument (200-µL specimen volume)

1. Ensure that the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head is set up with the KingFisher™ Flex 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.

2. Ensure that the **MVP_2Wash_200_Flex** program has been downloaded from the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit product page at **https://www.thermofisher.com/order/catalog/product/A48383** and loaded onto the instrument.

Prepare the processing plates (200-µL specimen volume)

Note: Do not add reagents to wells that have no specimen or control.

Prepare the processing plates according to the following table. Cover the plates with a temporary seal (such as MicroAmp™ Clear Adhesive Film), then store at room temperature for up to 1 hour while you set up the Sample Plate.

Plate ID	Plate position	Plate type	Reagent	Volume per well
Wash Plate 1	2	KingFisher™ 96 Deep-Well Plate	Wash Solution	500 µL
Wash Plate 2	3		80% Ethanol solution	500 µL
Elution Plate	4		Elution Buffer	50 µL
Tip Comb Plate	5	Place a KingFisher™ Deep Well 96 Tip Comb in a KingFisher™ 96 KF microplate or equivalent plate[1]		

[1] See "Before you begin—Automated method" on page 10 for equivalent plates.

Prepare Binding Bead Mix (200-µL specimen volume)

Prepare the required amount of Binding Bead Mix on each day of use.

1. Vortex the Binding Beads to ensure 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]
Binding Solution	265 µL
Binding Beads	10 µL
Total volume per well	275 µL

[1] Include 10% overage when making the Binding Bead Mix for use with multiple extractions.

Note: The Binding Bead Mix has been shown to be stable for ≤8 hours at room temperature.

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

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

Prepare the Sample Plate (200-µL specimen volume)

1. Invert the Binding Bead Mix 5 times gently to mix, then add 275 µL to each specimen well and the Negative Control well in the Sample Plate (KingFisher™ 96 Deep-Well Plate).

   Note:
   - Do not add Binding Bead Mix or other reagents to wells that have no specimen or control.
   - To ensure even distribution of beads to all specimens or wells, invert the Binding Bead Mix frequently during pipetting. The Binding Bead Mix is viscous, so pipet slowly to ensure that the correct amount is added. DO NOT reuse pipette tips to add Binding Bead Mix to the specimens, because the high viscosity will cause the volume that is added to vary.

2. Add 200 µL of specimen to each specimen well.

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

3. Add 200 µL of nuclease-free water to the Negative Control well.

4. Add the following components to each specimen and Negative Control well.
   - Add 5 µL of Proteinase K.
   - (*Optional*) If using an extraction control, add the required volume. For more information about using an extraction control, see the assay documentation.

   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.

   Note: The Proteinase K and extraction control can be pre-mixed on each day of use, then kept on ice. Add the required volume of pre-mixed Proteinase K and extraction control to each specimen or Negative Control well of the Sample Plate.

   For example, if your assay requires 5 µL of the extraction control per extraction, add 10 µL of pre-mixed Proteinase K and extraction control to each well.

   Note: The Proteinase K and extraction control mix has been shown to be stable for ≤8 hours on ice.

Process the specimens (200-µL specimen volume)

1. Select the **MVP_2Wash_200_Flex** on the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head.

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

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

3. After the run is complete (~22 minutes after start), immediately remove the Elution Plate from the instrument, then cover the plate with MicroAmp™ Clear Adhesive Film.

   IMPORTANT! To prevent evaporation, seal the plate containing the eluate immediately.

   The specimens are eluted in 50 µL of Elution Buffer (see "Prepare the processing plates (200-µL specimen volume)" on page 12).

Note:

- If bead carryover is observed, place the Elution Plate on a magnetic stand to pellet the beads, then pipette the eluate to a new 96-well plate. Review the assay results to determine if re-extraction is required.
- To ensure reliable performance of the KingFisher™ Flex Magnetic Particle Processor, perform preventive maintenance as instructed by the manufacturer.

Place the Elution Plate on ice for immediate use.

Extract nucleic acid—Automated method (400-µL specimen volume)

Set up the instrument (400-µL specimen volume)

1. Ensure that the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head is set up with the KingFisher™ Flex 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.

2. Ensure that the **MVP_2Wash_400_Flex** program has been downloaded from the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit product page at **https://www.thermofisher.com/order/catalog/product/A48383** and loaded onto the instrument.

Prepare the processing plates (400-µL specimen volume)

Note: Do not add reagents to wells that have no specimen or control.

Prepare the processing plates according to the following table. Cover the plates with a temporary seal (such as MicroAmp™ Clear Adhesive Film), then store at room temperature for up to 1 hour while you set up the Sample Plate.

Plate ID	Plate position	Plate type	Reagent	Volume per well
Wash Plate 1	2	KingFisher™ 96 Deep-Well Plate	Wash Solution	1,000 µL
Wash Plate 2	3		80% Ethanol solution	1,000 µL
Elution Plate	4		Elution Buffer	50 µL
Tip Comb Plate	5	Place a KingFisher™ Deep Well 96 Tip Comb in a KingFisher™ 96 KF microplate or equivalent plate[1]		

[1] See "Before you begin—Automated method" on page 10 for equivalent plates.

Prepare Binding Bead Mix (400-µL specimen volume)

Prepare the required amount of Binding Bead Mix on each day of use.

1. Vortex the Binding Beads to ensure 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]
Binding Solution	530 µL
Binding Beads	20 µL
Total volume per well	550 µL

[1] Include 10% overage when making the Binding Bead Mix for use with multiple extractions.

Note: The Binding Bead Mix has been shown to be stable for ≤8 hours at room temperature.

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

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

Prepare the Sample Plate (400-µL specimen volume)

1. Invert the Binding Bead Mix 5 times gently to mix, then add 550 µL to each specimen well and the Negative Control well in the Sample Plate (KingFisher™ 96 Deep-Well Plate).

   Note:
   - Do not add Binding Bead Mix or other reagents to wells that have no specimen or control.
   - To ensure even distribution of beads to all specimens or wells, invert the Binding Bead Mix frequently during pipetting. The Binding Bead Mix is viscous, so pipet slowly to ensure that the correct amount is added. DO NOT reuse pipette tips to add Binding Bead Mix to the specimens, because the high viscosity will cause the volume that is added to vary.

2. Add 400 µL of specimen to each specimen well.

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

3. Add 400 µL of nuclease-free water to the Negative Control well.

4. Add the following components to each specimen and Negative Control well.
   - Add 10 µL of Proteinase K.
   - (*Optional*) If using an extraction control, add the required volume. For more information about using an extraction control, see the assay documentation.

   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.

   Note: The Proteinase K and extraction control can be pre-mixed on each day of use, then kept on ice. Add the required volume of pre-mixed Proteinase K and extraction control to each specimen or Negative Control well of the Sample Plate.

   For example, if your assay requires 10 µL of the extraction control per extraction, add 20 µL of pre-mixed Proteinase K and extraction control to each well.

   Note: The Proteinase K and extraction control mix has been shown to be stable for ≤8 hours on ice.

Process the specimens (400-µL specimen volume)

1. Select the **MVP_2Wash_400_Flex** on the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head.

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

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

3. After the run is complete (~24 minutes after start), immediately remove the Elution Plate from the instrument, then cover the plate with MicroAmp™ Clear Adhesive Film.

   IMPORTANT! To prevent evaporation, seal the plate containing the eluate immediately.

   The specimens are eluted in 50 µL of Elution Buffer (see "Prepare the processing plates (400-µL specimen volume)" on page 15).

Note:

- If bead carryover is observed, place the Elution Plate on a magnetic stand to pellet the beads, then pipette the eluate to a new 96-well plate. Review the assay results to determine if re-extraction is required.
- To ensure reliable performance of the KingFisher™ Flex Magnetic Particle Processor, perform preventive maintenance as instructed by the manufacturer.

Place the Elution Plate on ice for immediate use.

3 Extract nucleic acid from swab-based specimens (manual method)

Manual nucleic acid extraction can be performed using a specimen volume of 200 µL or 400 µL. See your assay documentation for specific specimen volume recommendations.

Before you begin—Manual method

IMPORTANT! The Binding Bead Mix is not compatible 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. This is not a cause for concern and does not negatively affect performance.

- Patient 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.
- Review your assay documentation to determine if an extraction control is recommended to verify the efficacy of the nucleic acid preparation. Follow the extraction control guidelines provided in the assay documentation.
- 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 per plate.
- Prepare fresh 80% ethanol solution using Fisher BioReagents™ Ethanol, Absolute, Molecular Biology Grade and nuclease-free water. Prepare sufficient volume for the required number of extractions plus 10% overage.

Specimen volume	Volume of 80% ethanol solution per extraction
200 µL	0.75 mL
400 µL	1.5 mL

- Note the following for all kit components and 80% ethanol solution: Open reagent bottles, or reagents poured in reagent reservoirs, have been shown to be stable for ≤2 hours at room temperature.
- Mark the Negative Control well on the Sample Plate and the Elution Plate.

Extract nucleic acid—Manual method (200-µL specimen volume)

Prepare Binding Bead Mix (200-µL specimen volume)

Prepare the required amount of Binding Bead Mix on each day of use.

1. Vortex the Binding Beads to ensure 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]
Binding Solution	265 µL
Binding Beads	10 µL
Total volume per well	275 µL

[1] Include 10% overage when making the Binding Bead Mix for use with multiple extractions.

Note: The Binding Bead Mix has been shown to be stable for ≤8 hours at room temperature.

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

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

Digest with Proteinase K (200-µL specimen volume)

This section provides volumes for the Sample Plate (KingFisher™ 96 Deep-Well Plate). Your plate layout will depend on the number of specimens you run.

1. Invert the Binding Bead Mix 5 times gently to mix, then add 275 µL to each specimen well and Negative Control well in the Sample Plate.

Note: To ensure even distribution of beads to all specimens or wells, invert the Binding Bead Mix frequently during pipetting. The Binding Bead Mix is viscous, so pipet slowly to ensure that the

correct amount is added. DO NOT reuse pipette tips to add Binding Bead Mix to the specimens, because the high viscosity will cause the volume that is added to vary.

2. Add 200 µL of specimen to each specimen well.

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

3. Add 200 µL of nuclease-free water to the Negative Control well.

4. Add the following components to each well, including the Negative Control well.
   - Add 5 µL of Proteinase K.
   - (*Optional*) If using an extraction control, add the required volume. For more information about using an extraction control, see the assay documentation.

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.

Note: The Proteinase K and extraction control can be pre-mixed on each day of use, then kept on ice. Add the required volume of pre-mixed Proteinase K and extraction control to each well of the Sample Plate.

For example, if your assay requires 5 µL of the extraction control per extraction, add 10 µL of pre-mixed Proteinase K and extraction control to each well.

Note: The Proteinase K and extraction control mix has been shown to be stable for ≤8 hours on ice.

5. Seal the plate with MicroAmp™ Clear Adhesive Film, then shake the sealed plate at 1,050 rpm for 2 minutes.

6. Incubate the sealed plate at 65°C for 5 minutes (ensure the bottom of the plate is uncovered), then shake the plate at 1,050 rpm for 5 minutes.

7. Place the sealed plate on the magnetic stand for 10 minutes or until all of the beads have collected.

Wash the beads (200-µL specimen volume)

1. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

IMPORTANT! Avoid disturbing the beads.

2. Remove the plate from the magnetic stand, then add 500 µL of Wash Solution to each specimen.

3. Pipet up and down to mix the beads.

4. Reseal the plate, then shake at 1,050 rpm for 1 minute.

5. Place the plate back on the magnetic stand for 2 minutes, or until all the beads have collected.
6. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

   IMPORTANT! Avoid disturbing the beads.

7. Repeat step 2 to step 6 using 500 µL of 80% ethanol solution.
8. Repeat step 2 to step 6 using 250 µL of 80% ethanol solution.
9. If there is any ethanol solution remaining in the wells, use a 20-µL multichannel pipette to remove it.
10. Dry the beads by shaking the plate (uncovered) at 1,050 rpm for 2 minutes.

Elute the nucleic acid (200-µL specimen volume)

1. Add 50 µL of Elution Buffer to each specimen, then seal the plate with MicroAmp™ Clear Adhesive Film.
2. Shake the sealed plate at 1,050 rpm for 5 minutes.
3. Place the plate in an incubator at 65°C for 10 minutes.
4. Remove the plate from the incubator, then shake the plate at 1,050 rpm for 5 minutes.
5. Place the sealed plate on the magnetic stand for 3 minutes or until clear to collect the beads against the magnets.
6. Keeping the plate on the magnet, carefully remove the seal, transfer the eluates to a fresh standard (not deep-well) 96-well plate, then seal the plate with MicroAmp™ Clear Adhesive Film.

   IMPORTANT! To prevent evaporation, seal the plate containing the eluate immediately after the transfers are complete.

Note: If bead carryover is observed, place the Elution Plate on a magnetic stand to pellet the beads, then pipette the eluate to a new 96-well plate. Review the assay results to determine if re-extraction is required.

Place the Elution Plate on ice for immediate use.

Extract nucleic acid—Manual method (400-µL specimen volume)

Prepare Binding Bead Mix (400-µL specimen volume)

Prepare the required amount of Binding Bead Mix on each day of use.

1. Vortex the Binding Beads to ensure 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]
Binding Solution	530 µL
Binding Beads	20 µL
Total volume per well	550 µL

[1] Include 10% overage when making the Binding Bead Mix for use with multiple extractions.

Note: The Binding Bead Mix has been shown to be stable for ≤8 hours at room temperature.

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

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

Digest with Proteinase K (400-µL specimen volume)

This section provides volumes for the Sample Plate (KingFisher™ 96 Deep-Well Plate). Your plate layout will depend on the number of specimens you run.

1. Invert the Binding Bead Mix 5 times gently to mix, then add 550 µL to each specimen well and Negative Control well in the Sample Plate.

Note: To ensure even distribution of beads to all specimens or wells, invert the Binding Bead Mix frequently during pipetting. The Binding Bead Mix is viscous, so pipet slowly to ensure that the correct amount is added. DO NOT reuse pipette tips to add Binding Bead Mix to the specimens, because the high viscosity will cause the volume that is added to vary.

2. Add 400 µL of specimen to each specimen well.

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

3. Add 400 µL of nuclease-free water to the Negative Control well.

4. Add the following components to each well, including the Negative Control well.
   - Add 10 µL of Proteinase K.
   - (*Optional*) If using an extraction control, add the required volume. For more information about using an extraction control, see the assay documentation.

   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.

   Note: The Proteinase K and extraction control can be pre-mixed on each day of use, then kept on ice. Add the required volume of pre-mixed Proteinase K and extraction control to each well of the Sample Plate.
   For example, if your assay requires 10 µL of the extraction control per extraction, add 20 µL of pre-mixed Proteinase K and extraction control to each well.

   Note: The Proteinase K and extraction control mix has been shown to be stable for ≤8 hours on ice.

5. Seal the plate with MicroAmp™ Clear Adhesive Film, then shake the sealed plate at 1,050 rpm for 2 minutes.
6. Incubate the sealed plate at 65°C for 10 minutes (ensure the bottom of the plate is uncovered), then shake the plate at 1,050 rpm for 5 minutes.
7. Place the sealed plate on the magnetic stand for 10 minutes or until all of the beads have collected.

Wash the beads (400-µL specimen volume)

1. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

   IMPORTANT! Avoid disturbing the beads.

2. Remove the plate from the magnetic stand, then add 1 mL of Wash Solution to each specimen.
3. Pipet up and down to mix the beads.
4. Reseal the plate, then shake at 1,050 rpm for 1 minute.
5. Place the plate back on the magnetic stand for 2 minutes, or until all the beads have collected.
6. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

   IMPORTANT! Avoid disturbing the beads.

7. Repeat step 2 to step 6 using 1 mL of 80% ethanol solution.
8. Repeat step 2 to step 6 using 500 µL of 80% ethanol solution.

9. If there is any ethanol solution remaining in the wells, use a 20-µL multichannel pipette to remove it.

10. Dry the beads by shaking the plate (uncovered) at 1,050 rpm for 2 minutes.

Elute the nucleic acid (400-µL specimen volume)

1. Add 50 µL of Elution Buffer to each specimen, then seal the plate with MicroAmp™ Clear Adhesive Film.

2. Shake the sealed plate at 1,050 rpm for 5 minutes.

3. Place the plate in an incubator at 65°C for 10 minutes.

4. Remove the plate from the incubator, then shake the plate at 1,050 rpm for 5 minutes.

5. Place the sealed plate on the magnetic stand for 3 minutes or until clear to collect the beads against the magnets.

6. Keeping the plate on the magnet, carefully remove the seal, transfer the eluates to a fresh standard (not deep-well) 96-well plate, then seal the plate with MicroAmp™ Clear Adhesive Film.

   IMPORTANT! To prevent evaporation, seal the plate containing the eluate immediately after the transfers are complete.

Note: If bead carryover is observed, place the Elution Plate on a magnetic stand to pellet the beads, then pipette the eluate to a new 96-well plate. Review the assay results to determine if re-extraction is required.

Place the Elution Plate on ice for immediate use.

4 Performance characteristics

Analytical performance of the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit was evaluated using nasopharyngeal (NP) swab specimens and saliva specimens prepared using the kit and then analyzed using qPCR-based assays specific for viral targets. All studies are described in the following sections.

Note: Performance evaluation of the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit does not include validation of its use with any specific assay. Refer to your assay documentation for compatibility with this kit.

Analytical sensitivity

Analytical sensitivity of the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit was established as follows:

- Representative RNA viruses: The lowest viral concentration of SARS-CoV-2 virus, influenza A virus, influenza B virus, RSV A virus, or RSV B virus that can be extracted and detected at least 95% of the time was determined using the TaqPath™ COVID-19, Flu A/B, RSV Combo Kit.
- Representative DNA virus: The lowest viral concentration of adenovirus that can be extracted and detected at least 95% of the time was determined using a TaqMan™ adenovirus assay.

Analytical sensitivity—Nasopharyngeal specimens with representative RNA viruses

Negative nasopharyngeal (NP) swab specimens were pooled and spiked with the indicated strains of SARS-CoV-2 virus, influenza A virus, influenza B virus, RSV A virus, or RSV B virus at several concentrations. The specimens were extracted using the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit and analyzed using the TaqPath™ COVID-19, Flu A/B, RSV Combo Kit on the 7500 Fast Real-Time PCR Instrument. A three-phase approach was used to determine the limit of detection (LoD) for each virus. The preliminary LoD was established in phases I and II and confirmed in phase III by testing 20 replicates.

Table 1 LoD determination in NP specimens spiked with gamma-irradiated SARS-CoV-2 isolate USA-WA1/2020 (7500 Fast Real-Time PCR Instrument)

Concentration	Replicate	Mean C_t		Interpretation	% Positive
		C19	MS2		
50 GCE/mL	1	34.36	23.50	Positive	100%
	2	33.75	23.67	Positive	
	3	33.89	23.87	Positive	
	4	35.55	23.99	Positive	
	5	33.37	23.85	Positive	
	6	33.84	24.02	Positive	
	7	34.24	24.33	Positive	
	8	33.91	23.82	Positive	
	9	33.19	23.63	Positive	
	10	34.26	24.22	Positive	
	11	33.21	23.97	Positive	
	12	34.24	24.01	Positive	
	13	34.91	24.59	Positive	
	14	33.97	23.91	Positive	
	15	34.45	24.15	Positive	
	16	34.09	24.53	Positive	
	17	33.70	24.26	Positive	
	18	34.68	24.03	Positive	
	19	34.95	24.15	Positive	
	20	34.52	24.15	Positive	

Table 2 LoD determination in NP specimens spiked with live influenza A virus strain A/Perth/16/2009 (H3N2) (7500 Fast Real-Time PCR Instrument)

Concentration	Replicate	Mean C_t		Interpretation	% Positive
		Flu A/B	MS2		
350 GCE/mL	1	34.40	23.79	Positive	100%
	2	33.38	23.59	Positive	
	3	34.80	23.66	Positive	
	4	36.60	24.03	Positive	
	5	34.44	23.91	Positive	
	6	34.63	24.10	Positive	
	7	34.33	24.00	Positive	
	8	34.30	23.76	Positive	
	9	35.28	24.12	Positive	
	10	34.87	24.18	Positive	
	11	34.28	23.78	Positive	
	12	34.39	23.93	Positive	
	13	34.96	23.90	Positive	
	14	35.82	23.94	Positive	
	15	36.55	23.86	Positive	
	16	35.30	23.89	Positive	
	17	33.64	23.83	Positive	
	18	34.65	23.96	Positive	
	19	34.61	23.94	Positive	
	20	35.40	23.84	Positive	

Table 3 LoD determination in NP specimens spiked with live influenza A virus strain A/Brisbane/59/2007 (H1N1) (7500 Fast Real-Time PCR Instrument)

Concentration	Replicate	Mean C_t		Interpretation	% Positive
		Flu A/B	MS2		
384 GCE/mL	1	33.50	23.64	Positive	95%
	2	36.90	23.76	Positive	
	3	33.94	23.85	Positive	
	4	34.01	23.84	Positive	
	5	Undetermined	23.88	Negative	
	6	36.86	24.07	Positive	
	7	34.59	24.25	Positive	
	8	33.42	23.92	Positive	
	9	35.27	23.94	Positive	
	10	36.67	24.32	Positive	
	11	34.93	23.96	Positive	
	12	34.48	23.99	Positive	
	13	35.25	24.06	Positive	
	14	37.78	24.11	Positive	
	15	34.21	24.07	Positive	
	16	35.26	24.25	Positive	
	17	36.48	24.26	Positive	
	18	36.08	24.11	Positive	
	19	35.56	24.09	Positive	
	20	34.89	24.23	Positive	

Table 4 LoD determination in NP specimens spiked with live influenza B virus strain B/Florida/04/2006 (Yamagata lineage) (7500 Fast Real-Time PCR Instrument)

Concentration	Replicate	Mean C_t		Interpretation	% Positive
		Flu A/B	MS2		
1,250 GCE/mL	1	34.32	23.40	Positive	95%
	2	34.33	23.70	Positive	
	3	33.55	23.84	Positive	
	4	34.64	23.94	Positive	
	5	36.47	23.85	Positive	
	6	34.82	23.93	Positive	
	7	35.00	24.24	Positive	
	8	34.98	23.84	Positive	
	9	35.27	23.50	Positive	
	10	Undetermined	24.08	Negative	
	11	34.58	23.90	Positive	
	12	35.60	23.68	Positive	
	13	34.58	24.15	Positive	
	14	34.16	23.74	Positive	
	15	34.73	23.81	Positive	
	16	34.86	24.14	Positive	
	17	35.84	24.12	Positive	
	18	34.77	23.79	Positive	
	19	33.51	23.83	Positive	
	20	34.81	24.04	Positive	

Table 5 LoD determination in NP specimens spiked with live influenza B virus strain B/Wisconsin/1/2010 (Yamagata lineage) (7500 Fast Real-Time PCR Instrument)

Concentration	Replicate	Mean C_t		Interpretation	% Positive
		Flu A/B	MS2		
350 GCE/mL	1	35.32	23.33	Positive	95%
	2	34.06	23.56	Positive	
	3	33.88	23.71	Positive	
	4	34.66	23.75	Positive	
	5	34.73	23.31	Positive	
	6	36.46	23.64	Positive	
	7	34.09	23.78	Positive	
	8	36.07	23.92	Positive	
	9	35.09	23.62	Positive	
	10	35.12	23.64	Positive	
	11	35.30	23.71	Positive	
	12	Undetermined	23.91	Negative	
	13	34.95	23.76	Positive	
	14	35.37	23.54	Positive	
	15	34.37	23.50	Positive	
	16	34.88	23.93	Positive	
	17	35.79	23.76	Positive	
	18	34.63	23.83	Positive	
	19	35.24	23.62	Positive	
	20	35.20	23.95	Positive	

Table 6 LoD determination in NP specimens spiked with RSV A/2006 (7500 Fast Real-Time PCR Instrument)

Concentration	Replicate	Mean C_t		Interpretation	% Positive
		RSV	MS2		
200 GCE/mL	1	45.31	24.03	Negative	95%
	2	34.74	23.76	Positive	
	3	34.43	24.23	Positive	
	4	33.53	24.17	Positive	
	5	36.09	24.01	Positive	
	6	35.51	23.98	Positive	
	7	34.92	24.54	Positive	
	8	34.47	23.95	Positive	
	9	33.53	24.06	Positive	
	10	34.17	24.28	Positive	
	11	34.18	23.90	Positive	
	12	35.31	23.99	Positive	
	13	35.82	24.29	Positive	
	14	36.32	23.97	Positive	
	15	33.00	23.94	Positive	
	16	34.80	24.24	Positive	
	17	34.86	24.13	Positive	
	18	33.18	23.90	Positive	
	19	32.01	24.23	Positive	
	20	33.85	24.09	Positive	

Table 7 LoD determination in NP specimens spiked with RSV B/3/2015 Isolate #2 (7500 Fast Real-Time PCR Instrument)

Concentration	Replicate	Mean C_t		Interpretation	% Positive
		RSV	MS2		
200 GCE/mL	1	33.22	23.67	Positive	95%
	2	36.04	23.81	Positive	
	3	35.56	23.62	Positive	
	4	30.59	23.82	Positive	
	5	35.05	23.84	Positive	
	6	36.70	23.89	Positive	
	7	34.13	23.62	Positive	
	8	33.06	23.89	Positive	
	9	35.16	23.72	Positive	
	10	37.47	23.83	Positive	
	11	36.15	23.73	Positive	
	12	35.06	23.87	Positive	
	13	37.92	23.82	Positive	
	14	42.94	23.71	Positive	
	15	42.90	23.67	Positive	
	16	35.22	23.76	Positive	
	17	35.67	23.74	Positive	
	18	39.47	23.65	Positive	
	19	Undetermined	23.88	Negative	
	20	37.67	23.77	Positive	

Table 8 LoD results

Virus	Limit of Detection (7500 Fast Real-Time PCR Instrument)	
SARS-CoV-2 isolate USA-WA1/2020	50 GCE/mL	8.24×10^{-3} $TCID_{50}$/mL
Influenza A virus strain A/Perth/16/2009 (H3N2)	350 GCE/mL	2.21×10^{-2} $TCID_{50}$/mL
Influenza A virus strain A/Brisbane/59/2007 (H1N1)	384 GCE/mL	1.23×10^{-3} $TCID_{50}$/mL
Influenza B virus strain B/Florida/04/2006 (Yamagata lineage)	1,250 GCE/mL	1.47×10^{-1} $TCID_{50}$/mL
Influenza B virus strain B/Wisconsin/1/2010 (Yamagata lineage)	350 GCE/mL	4.24×10^{-3} $TCID_{50}$/mL
RSV A/2006	200 GCE/mL	1.36×10^{-2} $TCID_{50}$/mL
RSV B/3/2015 Isolate #2	200 GCE/mL	1.31×10^{-2} $TCID_{50}$/mL

Analytical sensitivity—Nasopharyngeal specimens with a representative DNA virus

Nasopharyngeal (NP) swab specimens that were negative for adenovirus were spiked with live human adenovirus 1 culture at several concentrations. The specimens were extracted using the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit and detected using a TaqMan™ adenovirus assay. The LoD was confirmed by testing 20 replicates.

Table 9 LoD determination in NP specimens spiked with adenovirus (Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument with 96-well block)

Effective concentration	TCID50/reaction	Replicate	Mean C_t	Interpretation	% Positive
5 TCID50/mL	0.1 TCID50/reaction	1	34.72	Positive	100%
		2	35.65	Positive	
		3	35.96	Positive	
		4	35.01	Positive	
		5	35.35	Positive	
		6	34.94	Positive	
		7	34.93	Positive	
		8	34.61	Positive	
		9	35.80	Positive	

Table 9 LoD determination in NP specimens spiked with adenovirus (Applied Biosystems QuantStudio 5 Real-Time PCR Instrument with 96-well block) *(continued)*

Effective concentration	TCID50/reaction	Replicate	Mean C_t	Interpretation	% Positive
5 TCID50/mL	0.1 TCID50/reaction	10	34.73	Positive	100%
		11	33.83	Positive	
		12	34.57	Positive	
		13	35.81	Positive	
		14	34.63	Positive	
		15	33.77	Positive	
		16	34.53	Positive	
		17	34.89	Positive	
		18	33.89	Positive	
		19	34.96	Positive	
		20	34.46	Positive	

Table 10 LoD determination in NP specimens spiked with adenovirus (QuantStudio™ 5 Real-Time PCR Instrument with 384-well block)

Effective concentration	TCID50/reaction	Replicate	Mean C_t	Interpretation	% Positive
5 TCID50/mL	0.1 TCID50/reaction	1	34.56	Positive	100%
		2	35.22	Positive	
		3	34.86	Positive	
		4	34.74	Positive	
		5	35.26	Positive	
		6	34.53	Positive	
		7	33.64	Positive	
		8	34.18	Positive	
		9	34.75	Positive	
		10	35.80	Positive	
		11	33.80	Positive	
		12	33.83	Positive	
		13	34.69	Positive	

Table 10 LoD determination in NP specimens spiked with adenovirus (QuantStudio 5 Real-Time PCR Instrument with 384-well block) *(continued)*

Effective concentration	TCID50/reaction	Replicate	Mean C_t	Interpretation	% Positive
5 TCID50/mL	0.1 TCID50/reaction	14	34.31	Positive	100%
		15	34.08	Positive	
		16	33.22	Positive	
		17	34.43	Positive	
		18	34.43	Positive	
		19	34.50	Positive	
		20	33.89	Positive	

Analytical sensitivity—Saliva specimens with a representative RNA virus

Negative saliva specimens were pooled and spiked with gamma-irradiated SARS-CoV-2 isolate USAWA1/2020 at several concentrations. The specimens were extracted using the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit on a KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head and analyzed using the TaqPath™ COVID-19 Combo Kit on a QuantStudio™ 5 Real-Time PCR Instrument (96-well and 384-well blocks). A three-phase approach was used to determine the limit of detection (LoD) for each virus. The preliminary LoD was established in phases I and II and confirmed in phase III by testing 20 replicates.

Table 11 LoD determination in saliva specimens spiked with SARS-CoV-2 (QuantStudio™ 5 Real-Time PCR Instrument—96-well block)

Concentration	Replicate	Mean C_t				Interpretation	% Positive
		S gene	ORF1ab	N gene	MS2		
125 GCE/mL	1	40.0	30.8	32.1	23.3	Positive	100%
	2	40.0	31.6	36.4	23.3	Positive	
	3	37.6	31.4	32.9	23.2	Positive	
	4	40.0	32.2	33.9	23.3	Positive	
	5	40.0	31.5	32.9	23.2	Positive	
	6	40.0	32.8	32.8	23.4	Positive	
	7	40.0	31.5	34.0	23.0	Positive	
	8	40.0	30.8	33.2	23.3	Positive	
	9	33.7	31.6	33.3	23.3	Positive	
	10	40.0	31.9	36.6	23.2	Positive	

Table 11 LoD determination in saliva specimens spiked with SARS-CoV-2 (QuantStudio 5 Real-Time PCR Instrument—96-well block) *(continued)*

Concentration	Replicate	Mean C_t				Interpretation	% Positive
		S gene	ORF1ab	N gene	MS2		
125 GCE/mL	11	40.0	31.8	34.4	23.3	Positive	100%
	12	40.0	31.2	33.3	23.1	Positive	
	13	40.0	30.8	33.5	23.5	Positive	
	14	40.0	32.0	33.0	23.6	Positive	
	15	40.0	31.5	34.9	23.4	Positive	
	16	40.0	30.9	33.5	23.3	Positive	
	17	37.3	31.4	34.2	23.3	Positive	
	18	29.7	29.2	30.7	23.3	Positive	
	19	40.0	31.6	33.3	23.3	Positive	
	20	38.2	31.6	33.2	23.6	Positive	

Table 12 LoD determination in saliva specimens spiked with SARS-CoV-2 (QuantStudio™ 5 Real-Time PCR Instrument—384-well block)

Concentration	Replicate	Mean C_t				Interpretation	% Positive
		S gene	ORF1ab	N gene	MS2		
250 GCE/mL	1	33.3	31.8	33.6	24.3	Positive	100%
	2	34.9	31.4	33.9	24.0	Positive	
	3	32.7	30.3	32.2	23.7	Positive	
	4	30.9	27.1	32.3	23.2	Positive	
	5	30.1	28.6	32.4	23.3	Positive	
	6	38.3	30.0	32.3	23.6	Positive	
	7	33.5	30.5	31.7	23.5	Positive	
	8	39.0	30.7	32.5	23.6	Positive	
	9	28.6	29.0	32.2	23.4	Positive	
	10	32.3	30.9	33.2	24.0	Positive	
	11	40.0	32.7	35.3	24.2	Positive	
	12	34.7	31.9	33.5	24.2	Positive	
	13	40.0	31.9	36.0	24.2	Positive	

Table 12 LoD determination in saliva specimens spiked with SARS-CoV-2 (QuantStudio 5 Real-Time PCR Instrument—384-well block) *(continued)*

Concentration	Replicate	Mean C_t				Interpretation	% Positive
		S gene	ORF1ab	N gene	MS2		
250 GCE/mL	14	34.3	32.3	32.7	24.1	Positive	100%
	15	32.4	29.9	33.8	23.6	Positive	
	16	29.6	27.3	32.4	23.5	Positive	
	17	32.4	29.5	32.1	23.7	Positive	
	18	32.7	30.7	32.4	23.7	Positive	
	19	33.7	30.9	33.4	23.6	Positive	
	20	32.9	29.4	32.7	23.3	Positive	

Precision study

A study was performed to characterize the reproducibility and precision of the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit when extracting RNA and DNA from nasopharyngeal (NP) and saliva specimens.

Three lots of MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit reagents were used for this study, and isolations were performed on 3 different KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head instruments for each specimen type at each input at each volume. Each lot was run on a separate instrument. At least 24 extraction replicates were generated per run for each LoD level (2X and 10X), for a total of 72 extraction replicates per LoD level for each viral target and sample type. The TaqPath™ COVID-19, Flu A/B, RSV Combo Kit was used to test nucleic acid extracted from nasopharyngeal (NP) and saliva samples for SARS-CoV-2 (a representive RNA virus), and a TaqMan™ adenovirus assay was used to test nucleic acid extracted from NP samples for adenovius (a representive DNA virus). The data below demonstrate intra-run and inter-run precision. All runs were positive for the viral target, and the coefficient of variation for the C_t value was shown to be <4% across all targets, LoD inputs, and sample types.

Intra-run, inter-run, and lot-to-lot repeatability

Note: Intra-run is defined as variability within a given run, on the same instrument, using the same lot of material.

Table 13 Intra-run repeatability—SARS-CoV-2 in nasopharyngeal swab samples

Sample Volume	LoD	GCE/mL	Mean C_t	SD	CV
Intra-run precision, Run 1/Lot 1					
200 µL	2X	100	31.46	0.46	1.46
	10X	500	28.99	0.25	0.86
400 µL	2X	100	31.59	0.38	1.21
	10X	500	29.06	0.19	0.66
Intra-run precision, Run 2/Lot 2					
200 µL	2X	100	31.77	0.41	1.28
	10X	500	29.20	0.16	0.55
400 µL	2X	100	31.95	0.32	1.01
	10X	500	29.32	0.14	0.47
Intra-run precision, Run 3/Lot 3					
200 µL	2X	100	32.28	0.39	1.20
	10X	500	29.58	0.19	0.65
400 µL	2X	100	31.95	0.35	1.10
	10X	500	29.12	0.20	0.70

Table 14 Intra-run repeatability, SARS-CoV-2 in saliva samples

LoD	GCE/mL	Mean C_t	SD	CV
Intra-run precision, Run 1/Lot 1				
2X	100	31.72	0.98	3.08
10X	500	29.24	0.21	0.71
Intra-run precision, Run 2/Lot 2				
2X	100	32.52	0.64	1.98
10X	500	29.51	0.20	0.68

Table 14 Intra-run repeatability, SARS-CoV-2 in saliva samples *(continued)*

LoD	GCE/mL	Mean C_t	SD	CV
Intra-run precision, Run 3/Lot 3				
2X	100	32.04	0.76	2.37
10X	500	29.36	0.24	0.81

Table 15 Intra-run repeatability, adenovirus in nasopharyngeal swab samples

Sample Volume	LoD	TCID50/mL	Mean C_t	SD	CV
Intra-run precision, Run 1/Lot 1					
200 µL	2X	10	32.18	0.37	1.16
	10X	50	30.36	0.42	1.37
400 µL	2X	10	33.93	0.46	1.35
	10X	50	31.90	0.28	0.87
Intra-run precision, Run 2/Lot 2					
200 µL	2X	10	33.07	0.57	1.71
	10X	50	30.99	0.71	2.30
400 µL	2X	10	34.25	0.45	1.33
	10X	50	32.23	0.33	1.03
Intra-run precision, Run 3/Lot 3					
200 µL	2X	10	33.18	0.29	0.86
	10X	50	31.17	0.26	0.83
400 µL	2X	10	34.15	0.35	1.01
	10X	50	32.19	0.25	0.77

Inter-run repeatability was measured across all runs. Each run utilized one lot of material.

Table 16 Inter-run repeatability, SARS-CoV-2 in nasopharyngeal swab samples

Sample Volume	LoD	GCE/mL	Mean C_t	SD	CV
200 µL	2X	100	31.83	0.54	1.69
	10X	500	29.26	0.32	1.09
400 µL	2X	100	31.83	0.39	1.22
	10X	500	29.17	0.21	0.71

Table 17 Inter-run repeatability, SARS-CoV-2 in saliva samples

Sample Volume	LoD	GCE/mL	Mean C_t	SD	CV
200 µL	2X	100	32.06	0.86	2.70
	10X	500	29.25	1.03	3.52

Table 18 Inter-run repeatability, adenovirus in nasopharyngeal swab samples

Sample Volume	LoD	TCID50/mL	Mean C_t	SD	CV
200 µL	2X	10	32.79	0.62	1.88
	10X	50	30.74	0.87	2.84
400 µL	2X	10	33.79	0.42	1.25
	10X	50	31.13	0.34	1.08

Nucleic acid yield from samples in the study

The primary source of nucleic acid in a sample is endogenous nucleic acid from human cells. Because microbial nucleic acid represents only a small fraction of the total yield from an extraction, the differences in the viral target nucleic acid extracted from 2X or 10X LoD input are only discernible through target-specific qPCR assays. The following tables show the total nucleic acid yield from the samples in the study as measured by the NanoDrop™ 8000 Spectrophotometer. All samples in the study produced an expected call of "positive", demonstrating that the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit produces nucleic acid of sufficient yield and quality for use in viral target-specific qPCR assays.

Table 19 DNA yield in nasopharyngeal swab samples as measured by the NanoDrop™ 8000 Spectrophotometer

Sample volume	LoD	Concentration (ng/µL)[1]
Run 1		
200 µL	2X	9.5
	10X	8.8
400 µL	2X	14.3
	10X	13.3
Run 2		
200 µL	2X	8.9
	10X	9.3
400 µL	2X	12.8
	10X	13.2

Table 19 DNA yield in nasopharyngeal swab samples as measured by the NanoDrop 8000 Spectrophotometer *(continued)*

Sample volume	LoD	Concentration (ng/µL)[1]
Run 3		
200 µL	2X	12.3
	10X	11.2
400 µL	2X	12.7
	10X	10.7

[1] Number of samples (n) = 48 for each input volume/contrived LoD

Table 20 RNA yield in nasopharyngeal swab samples as measured by the NanoDrop™ 8000 Spectrophotometer

Matrix input volume	Contrived LoD	Concentration (ng/µL)[1]
Run 1		
200 µL	2X	8.8
	10X	8.5
400 µL	2X	11.9
	10X	10.9
Run 2		
200 µL	2X	6.9
	10X	7.2
400 µL	2X	10.9
	10X	11.2
Run 3		
200 µL	2X	6.8
	10X	6.6
400 µL	2X	10.3
	10X	10.8

[1] Number of samples (n) = 48 for each input volume/contrived LoD

Table 21 RNA yield in saliva samples as measured by the NanoDrop™ 8000 Spectrophotometer

Matrix input volume	Contrived LoD	Concentration (ng/µL)[1]
Run 1		
200 µL	2X	87.8
	10X	83.5
Run 2		
200 µL	2X	77.2
	10X	71.4
Run 3		
200 µL	2X	81.8
	10X	79.3

[1] Number of samples (n) = 48 for each input volume/contrived LoD

Interfering substances

Pooled negative NP specimens were spiked with SARS-CoV-2, influenza A virus, influenza B virus, RSV A virus, or RSV B virus at 3X LoD, extracted using the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit, and run in triplicate for each of the 11 potentially interfering substances and one no-interferent control using the TaqPath™ COVID-19, Flu A/B, RSV Combo Kit on the 7500 Fast Real-Time PCR Instrument.

Interference was not observed for mucin, blood, corticosteroid nasal spray, nasal gel, homeopathic allergy relief nasal spray, throat lozenges, Oseltamivir, antibiotic ointment, and systemic antibiotics. Afrin™ Original nasal spray showed interference at 10% v/v. The highest concentrations that did not produce interference for any viral target was 0.63%.

Table 22 Interfering substances

Interfering substance	Final concentration in sample	Agreement with expected results				
		SARS-CoV-2	Flu A	Flu B	RSV A	RSV B
None	N/A	100%	100%	100%	100%	100%
Mucin: bovine submaxillary gland, type I-S	0.1 mg/mL	100%	100%	100%	100%	100%
Blood (human)	1% v/v	100%	100%	100%	100%	100%
Nasal sprays or drops —Afrin™ Original	0.6% v/v	100%[1]	100%[2]	100%[2]	100%[2]	100%[2]

Table 22 Interfering substances *(continued)*

Interfering substance	Final concentration in sample	Agreement with expected results				
		SARS-CoV-2	Flu A	Flu B	RSV A	RSV B
Nasal corticosteroids —Flonase™	5 µg/mL	100%	100%	100%	100%	100%
Nasal gel—NeilMed™ Nasogel™	1% w/v	100%	100%	100%	100%	100%
Homeopathic allergy relief medicine—NatraBio Allergy Relief	10% v/v	100%	100%	100%	100%	100%
Throat lozenges, oral anesthetic and analgesic—Chloraseptic™	1% w/v	100%	100%	100%	100%	100%
Oseltamivir phosphate	33 µg/mL	100%	100%	100%	100%	100%
Antibiotic, nasal ointment—Teva™	5 µg/mL	100%	100%	100%	100%	100%
Antibacterial, systemic —Tobramycin	0.6 mg/mL	100%	100%	100%	100%	100%
Nasal sprays or drops —Neo-Synephrine	10% v/v	100%	100%	100%	100%	100%

[1] All replicates tested at 10%, 5%, and 2.5% v/v were undetected.

[2] All replicates tested at 10%, 5%, 2.5%, and 1.3% v/v were undetected.

A 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
  https://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 KingFisher™ purification system may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

Documentation and support

Related documentation

Document	Publication Number
MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit Supplemental Instructions	MAN0025302
Thermo Scientific™ KingFisher™ Flex User Manual	N07669

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

Limited product warranty

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