appliedbiosystems

Procedures for viral nucleic acid isolation USER BULLETIN

for 200 μ L or 400 μ L of sample

for use with MagMAX[™] Viral/Pathogen Nucleic Acid Isolation Kit or MagMAX[™] Viral/Pathogen II Nucleic Acid Isolation Kit

Publication Number MAN0019332

Revision D.0





Life Technologies Corporation | 2130 Woodward Street | Austin, TX 78744 For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0019332

Revision	Date	Description	
D.0	30 April 2021	Changed sku in required materials not supplied from A48383 to A48383R.	
C.0	02 September 2020	Added Important note in Before you begin topic.	
B.0	13 May 2020	Added the following products as an alternative to the KingFisher [™] 96 KF microplate for the tip comb plate:	
		Tip Comb Presenting Plate for KF 96	
		Nunc [™] MicroWell [™] 96-Well Microplate, barcoded	
		 Nunc[™] MicroWell[™] 96-Well Microplate, Flat Bottom 	
		Nunc [™] F96 MicroWell [™] Black Polystyrene Plate	
		KingFisher [™] Deepwell 96 Plate	
A.0	6 May 2020	New document.	

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

TRADEMARKS: All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.

©2021 Thermo Fisher Scientific Inc. All rights reserved.

Contents

CHAPTER 1	Product information	5
Prod	uct information	5
Requ	uired materials not supplied	5
CHAPTER 2	Extract RNA (automated method)	8
Befo	re you begin	8
Extra	act RNA—Automated method (200-µL sample input volume)	9
	Set up the instrument (200-µL sample input volume)	9
	Prepare the processing plates (200-µL sample input volume)	
	Prepare Binding Bead Mix (200-µL sample input volume)	10
	Prepare sample plate (200-µL sample input volume)	10
	Process the samples (200-μL sample input volume)	11
Extra	ct RNA-Automated method (400-μL sample input volume)	11
	Set up the instrument (400-µL sample input volume)	
	Prepare the processing plates (400-µL sample input volume)	
	Prepare Binding Bead Mix (400-µL sample input volume)	
	Prepare sample plate (400-µL sample input volume)	
	Process the samples (400-μL sample input volume)	13
CHAPTER 3	Extract RNA (manual method)	14
Befo	re you begin	
Extra	nct RNA-Manual method (200-μL sample input volume)	14
	Prepare Binding Bead Mix (200-µL sample input volume)	
	Digest with Proteinase K (200-μL sample input volume)	
	Wash the beads (200-µL sample input volume)	
	Elute the nucleic acid (200-µL sample input volume)	16
Extra	ct RNA-Manual method (400-μL sample input volume)	16
	Prepare Binding Bead Mix (400-µL sample input volume)	
	Digest with Proteinase K (400-μL sample input volume)	
	Wash the beads (400-μL sample input volume)	
	Elute the nucleic acid (400-µL sample input volume)	18

APPENDIX A	Safety	19
Chem	ical safety	. 20
Biolog	ical hazard safety	. 21
APPENDIX B	Documentation and support	. 22
Relate	d documentation	. 22
Custo	mer and technical support	. 22
Limite	d product warranty	. 22



Product information

Product information

This user bulletin describes the procedure to isolate viral and pathogen nucleic acid from 200 μ L or 400 μ L of sample with the following kits:

- MagMAX[™] Viral/Pathogen Nucleic Acid Isolation Kit
- MagMAX[™] Viral/Pathogen II Nucleic Acid Isolation Kit

Nucleic acid is isolated from upper respiratory specimens (such as nasopharyngeal, oropharyngeal, nasal, and mid-turbinate swabs, and nasopharyngeal aspirate) and bronchoalveolar lavage (BAL) specimens.

The procedures for automated and manual extractions are described. Automated extractions are performed with the KingFisher^{$^{\text{TM}}$} Flex Purification System (KingFisher).

The sample input volume is 200 μ L or 400 μ L.

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.

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

Chapter 1 Product information Required materials not supplied

(continued)

Item	Source		
96-well plate for the tip comb, one of the following:			
KingFisher [™] 96 KF microplate	• 97002540		
Tip Comb Presenting Plate for KF 96	• 267600		
 Nunc[™] MicroWell[™] 96-Well Microplate, Flat Bottom 	• 167008		
 Nunc[™] MicroWell[™] 96-Well Microplate, barcoded 	• 269787		
 ABgene[™] 96–Well Polypropylene Storage Microplate 	• AB0796		
 ABgene[™] 96–Well 1.2–mL Polypropylene Deepwell Storage Plate 	• AB1127		
 Nunc[™] F96 MicroWell[™] Black Polystyrene Plate 	• 137101		
 Nunc[™] F96 MicroWell[™] White Polystyrene Plate 	• 136101		
 KingFisher[™] Deepwell 96 Plate 	• 95040450, A48305, A48424, 95040455		
KingFisher [™] 96 tip comb for DW magnets	97002534, A48438, A48414		
Manual nucleic acid extraction system and materials			
	AM10027		
Magnetic Stand-96	AM10050		
Compact Digital Microplate Shaker	88882005		
Incubator capable of reaching 65°C with slatted shelves	MLS		
KingFisher [™] Deepwell 96 Plate	95040450, A48305, A48424, 95040455		
Standard 96-well plate for the eluate, one of the following:			
 KingFisher[™] 96 KF microplate 	• 97002540		
 MicroAmp[™] Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL 	• 4346906, 4366932		
 MicroAmp[™] Fast Optical 96-Well Reaction Plate, 0.1 mL 	• 4346907		
 MicroAmp[™] Optical 96-Well Reaction Plate with Barcode, 0.2 mL 	• 4306737, 4326659		
 MicroAmp[™] Optical 96-Well Reaction Plate, 0.2 mL 	• N8010560, 4316813		
MicroAmp [™] Clear Adhesive Film	4306311		
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	
Kits and reagents		
MagMAX [™] Viral/Pathogen Nucleic Acid Isolation Kit (up to 200 preparations, when 200 μL of sample is used)	A42352	
MagMAX [™] Viral/Pathogen Nucleic Acid Isolation Kit (up to 2,000 preparations, when 200 μL of sample is used)	A48310	
MagMAX [™] Viral/Pathogen II Nucleic Acid Isolation Kit (up to 2,000 preparations, when 200 μL of sample is used)	A48383R	
Fisher BioReagents [™] Ethanol, Absolute, Molecular Biology Grade ^[1] , or equivalent	BP2818100, BP2818500, BP28184	
Nuclease-free Water (not DEPC-Treated)	MLS	
Tubes, plates, and other consumables		
MicroAmp [™] Clear Adhesive Film	4306311	
MicroAmp [™] Adhesive Film Applicator	4333183	
Nonstick, RNase-free microcentrifuge tubes (1.5 mL and 2.0 mL)	thermofisher.com/plastics	
Sterile aerosol barrier (filtered) pipette tips	thermofisher.com/pipettetips	

^[1] Available at fisherscientific.com.



Extract RNA (automated method)

Before you begin	8
Extract RNA—Automated method (200-µL sample input volume)	9
Extract RNA—Automated method (400-uL sample input volume)	11

Automated RNA extraction is performed using the KingFisher[™] Flex Magnetic Particle Processor with 96 Deep-Well Head and the MagMAX[™] Viral/Pathogen Nucleic Acid Isolation Kit or MagMAX[™] Viral/Pathogen II Nucleic Acid Isolation Kit with a sample input volume of 200 µL or 400 µL.

Before you begin

IMPORTANT! Wash 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.

- Determine the number of required reactions based on the number of samples to be processed, plus one Negative Control per plate.
- Prepare fresh 80% Ethanol using Ethanol, Absolute, Molecular Biology Grade and Nuclease-free Water (not DEPC-Treated) for the required number of reactions, sufficient for 1 mL per reaction, plus 10% overage.
- Label the short side of each KingFisher[™] Deepwell 96 Plate (4):

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

Label the short side of the KingFisher[™] 96 KF microplate (1):

Label	Number of plates	
Tip comb	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[™] Deepwell 96 Plate
- Mark the Negative Control well on the plate.

Extract RNA—Automated method (200-µL sample input volume)

The following procedure uses components from the MagMAX[™] Viral/Pathogen Nucleic Acid Isolation Kit or the MagMAX[™] Viral/Pathogen II Nucleic Acid Isolation Kit.

Set up the instrument (200-µL sample input 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 product page and loaded onto the instrument.

Prepare the processing plates (200-µL sample input volume)

Prepare the processing plates according to the following table. Cover the plates with a temporary seal (such as $MicroAmp^{TM}$ 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 1 Plate	2	KingFisher [™] Deepwell 96 Plate	Wash Buffer	500 μL
Wash 2 Plate	3		80% Ethanol	1,000 µL
Elution Plate	4		Elution Solution	50 μL
Tip Comb Plate	5	Place a KingFisher [™] 96 tip comb for DW magnets in a KingFisher [™] 96 KF microplate		

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[™] Deepwell 96 Plate

Prepare Binding Bead Mix (200-µL sample input volume)

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

- 1. Vortex the Total Nucleic Acid Magnetic Beads to ensure that the bead mixture is homogeneous.
- 2. For the number of required reactions, prepare the Binding Bead Mix according to the following table:

Component	Volume per well ^[1]
Binding Solution	265 μL
Total Nucleic Acid Magnetic Beads	10 μL
Total volume per well	275 μL

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

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

Prepare sample plate (200-µL sample input volume)

- 1. Add 5 µL of Proteinase K to each well in the KingFisher[™] Deepwell 96 Plate labeled "Sample Plate".
- 2. Add 200 µL of sample to each sample well.
- 3. Add 200 µL of Nuclease-free Water (not DEPC-Treated) to the Negative Control well.
- 4. Invert the Binding Bead Mix 5 times gently to mix, then add 275 μL to each sample well and the Negative Control well in the Sample Plate.

Note: Remix Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. 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 samples, as the high viscosity will cause variations in the volumes added.

5. Add 5 µL of MS2 Phage Control to each sample well and to the Negative Control well.

Process the samples (200-µL sample input volume)

- 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.
- 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 samples are eluted in 50 μ L of Elution Solution (see "Prepare the processing plates (200- μ L sample input volume)" on page 9).

Note: Significant bead carry over may adversely impact RT-PCR performance.

Place the Elution Plate on ice for immediate use in real-time RT-PCR.

Extract RNA—Automated method (400-µL sample input volume)

The following procedure uses components from the MagMAX[™] Viral/Pathogen Nucleic Acid Isolation Kit or the MagMAX[™] Viral/Pathogen II Nucleic Acid Isolation Kit.

Set up the instrument (400-µL sample input 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 product page and loaded onto the instrument.

Prepare the processing plates (400-µL sample input volume)

Prepare the processing plates according to the following table. Cover the plates with a temporary seal (such as $MicroAmp^{TM}$ 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 1 Plate	2		Wash Buffer	1,000 µL
Wash 2 Plate	3	KingFisher [™] Deepwell 96 Plate	80% Ethanol	1,000 µL
Elution Plate	4		Elution Solution	50 μL
Tip Comb Plate	5	Place a KingFisher [™] 96 tip comb for DW magnets in a KingFisher [™] 96 KF microplate		

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[™] Deepwell 96 Plate

Prepare Binding Bead Mix (400-µL sample input volume)

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

- 1. Vortex the Total Nucleic Acid Magnetic Beads to ensure that the bead mixture is homogeneous.
- 2. For the number of required reactions, prepare the Binding Bead Mix according to the following table:

Component	Volume per well ^[1]
Binding Solution	530 μL
Total Nucleic Acid Magnetic Beads	20 μL
Total volume per well	550 μL

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

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

Prepare sample plate (400-µL sample input volume)

- Add 10 µL of Proteinase K to each well in the KingFisher[™] Deepwell 96 Plate labeled "Sample Plate".
- 2. Add 400 µL of sample to each sample well.
- 3. Add 400 µL of Nuclease-free Water (not DEPC-Treated) to the Negative Control well.
- 4. Invert the Binding Bead Mix 5 times gently to mix, then add 550 μ L to each sample well and the Negative Control well 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 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 samples, as the high viscosity will cause variations in the volumes added.

5. Add 10 µL of MS2 Phage Control to each sample well and to the Negative Control well.

Process the samples (400-µL sample input volume)

- 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.
- 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 samples are eluted in 50 μ L Elution Solution (see "Prepare the processing plates (400- μ L sample input volume)" on page 12).

Note: Significant bead carry over may adversely impact RT-PCR performance.

Place the Elution Plate on ice for immediate use in real-time RT-PCR.



Extract RNA (manual method)

Before you begin	14
Extract RNA—Manual method (200-µL sample input volume)	14
Extract RNA—Manual method (400-µL sample input volume)	16

Manual RNA extraction can be performed from a sample input volume of 200 µL or 400 µL using either the MagMAX[™] Viral/Pathogen Nucleic Acid Isolation Kit or the MagMAX[™] Viral/Pathogen II Nucleic Acid Isolation Kit.

Before you begin

- Determine the number of required reactions based on the number of patient samples to be processed, plus one Negative Control per plate.
- Prepare fresh 80% Ethanol using Ethanol, Absolute, Molecular Biology Grade and Nuclease-free Water (not DEPC-Treated) for the required number of reactions, plus 10% overage.

Sample input volume	Volume of 80% Ethanol per reaction
200 μL	0.75 mL
400 μL	1.5 mL

• Mark the Negative Control well on the plate.

Extract RNA—Manual method (200-µL sample input volume)

The following procedure uses components from the MagMAX[™] Viral/Pathogen Nucleic Acid Isolation Kit or the MagMAX[™] Viral/Pathogen II Nucleic Acid Isolation Kit.

Prepare Binding Bead Mix (200-µL sample input volume)

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

- 1. Vortex the Total Nucleic Acid Magnetic Beads to ensure that the bead mixture is homogeneous.
- 2. For the number of required reactions, prepare the Binding Bead Mix according to the following table:

Component	Volume per well ^[1]
Binding Solution	265 μL
Total Nucleic Acid Magnetic Beads	10 μL
Total volume per well	275 μL

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

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

Digest with Proteinase K (200-µL sample input volume)

- 1. Add 5 µL of Proteinase K to each well of a KingFisher[™] Deepwell 96 Plate.
- 2. Add 200 µL of sample to each sample well.
- 3. Add 200 µL of Nuclease-free Water (not DEPC-Treated) to the Negative Control well.
- 4. Invert the Binding Bead Mix 5 times gently to mix, then add 275 μL to each sample well and Negative Control well.

Note: Remix the Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. 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 samples, as the high viscosity will cause variations in the volumes added.

- 5. Add 5 μL of MS2 Phage Control to each sample well and to the Negative Control well.
- **6.** Seal the plate with MicroAmp[™] Clear Adhesive Film, then shake the sealed plate at 1,050 rpm for 2 minutes.
- 7. 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.
- 8. Place the sealed plate on the magnetic stand for 10 minutes or until all of the beads have collected.

Wash the beads (200-µL sample input 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 Buffer to each sample.
- 3. Reseal the plate, then shake at 1,050 rpm for 1 minute.
- 4. Place the plate back on the magnetic stand for 2 minutes, or until all the beads have collected.
- 5. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

IMPORTANT! Avoid disturbing the beads.

- 6. Repeat step 2 to step 5 using 500 µL of 80% Ethanol.
- 7. Repeat step 2 to step 5 using 250 µL of 80% Ethanol.
- 8. Dry the beads by shaking the plate (uncovered) at 1,050 rpm for 2 minutes.

Elute the nucleic acid (200-µL sample input volume)

- Add 50 µL of Elution Solution to each sample, 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: Significant bead carry over may adversely impact RT-PCR performance.

Place the plate on ice for immediate use in real-time RT-PCR.

Extract RNA—Manual method (400-µL sample input volume)

The following procedure uses components from the MagMAX[™] Viral/Pathogen Nucleic Acid Isolation Kit or the MagMAX[™] Viral/Pathogen II Nucleic Acid Isolation Kit.

Prepare Binding Bead Mix (400-µL sample input volume)

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

- 1. Vortex the Total Nucleic Acid Magnetic Beads to ensure that the bead mixture is homogeneous.
- 2. For the number of required reactions, prepare the Binding Bead Mix according to the following table:

Component	Volume per well ^[1]
Binding Solution	530 μL
Total Nucleic Acid Magnetic Beads	20 μL
Total volume per well	550 μL

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

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

Digest with Proteinase K (400-µL sample input volume)

- 1. Add 10 µL of Proteinase K to each well of a KingFisher[™] Deepwell 96 Plate.
- 2. Add 400 µL of sample to each sample well.
- 3. Add 400 µL of Nuclease-free Water (not DEPC-Treated) to the Negative Control well.
- **4.** Invert the Binding Bead Mix 5 times gently to mix, then add 550 μL to each sample well and Negative Control well.

Note: Remix the Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. 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 samples, as the high viscosity will cause variations in the volumes added.

- 5. Add 10 µL of MS2 Phage Control to each sample well and to the Negative Control well.
- **6.** Seal the plate with MicroAmp[™] Clear Adhesive Film, then shake the sealed plate at 1,050 rpm for 2 minutes.
- 7. 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.
- 8. Place the sealed plate on the magnetic stand for 10 minutes or until all of the beads have collected.

Wash the beads (400-µL sample input 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 Buffer to each sample.
- 3. Reseal the plate, then shake at 1,050 rpm for 1 minute.
- 4. Place the plate back on the magnetic stand for 2 minutes, or until all the beads have collected.
- 5. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

IMPORTANT! Avoid disturbing the beads.

- 6. Repeat step 2 to step 5 using 1 mL of 80% Ethanol.
- 7. Repeat step 2 to step 5 using 500 µL of 80% Ethanol.
- 8. Dry the beads by shaking the plate (uncovered) at 1,050 rpm for 2 minutes.

Elute the nucleic acid (400-µL sample input volume)

- Add 50 µL of Elution Solution to each sample, 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: Significant bead carry over may adversely impact RT-PCR performance.

Place the plate on ice for immediate use in real-time RT-PCR.

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, see the "Documentation and Support" section in this document.

Appendix A Safety Chemical safety

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.



AVERTISSEMENT! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter:

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- · Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT!** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

Biological hazard safety



WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.



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.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
 - https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, Laboratory Biosafety Manual, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
 - www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf



Documentation and support

Related documentation

Document	Publication Number
MagMAX [™] Viral/Pathogen Nucleic Acid Isolation Kit (automated extraction) User Guide	MAN0018073
MagMAX [™] Viral/Pathogen Nucleic Acid Isolation Kit (manual extraction) User Guide	MAN0018072
Thermo Scientific [™] KingFisher [™] Flex User Manual	N07669

Customer and technical support

Visit thermofisher.com/support for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
 - Product FAQs
 - Software, patches, and updates
 - Training for many applications and instruments
- Order and web support
- 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.

