applied biosystems

MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit

USER GUIDE

Catalog Number A48383R

Publication Number MAN0024756

Revision A.0

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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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.

Revision	Date	Description
A.0	17 February 2021	New document for the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit (Cat. No. A48383R).

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1 Product information

Product description

The Applied Biosystems™ MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit (Cat. No. A48383R) is specifically designed for scalable, rapid purification of high quality nucleic acid (RNA and DNA) from virus in viral transport media (VTM) or saliva. The kit utilizes MagMAX™ magnetic-bead technology, ensuring reproducible recovery of high-quality nucleic acid for a range of downstream applications, such as sequencing and qPCR.

This product can be used with a manual or automated workflow. The automated workflow uses the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head to process 96 samples in <30 minutes.

Contents and storage

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

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	

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 **Note:** Includes the KingFisher™ Flex 96 Deep-Well Heating Block	5400630
KingFisher™ 96 Deep-Well Plate	95040450, A48305, A48424, 95040455
Use one of the following plates for the Tip Comb Plate: **IMPORTANT!** Use of alternate plates may misalign or damage the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head when following an automated protocol.	
• 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™ 96 Deep-Well Plate	• 95040450, A48305, A48424, 95040455
KingFisher™ 96 tip comb for DW magnets	97002534, A48438, A48414
(*Optional*) Magnetic Stand-96	AM10027 AM10050
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

(continued)

Item	Source
Use one of the following plates for the Elution Plate: **IMPORTANT!** Use of alternate plates may misalign or damage the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head when following an automated protocol.	
• 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
Reagents	
Fisher BioReagents™ Ethanol, Absolute, Molecular Biology Grade[1], or equivalent	BP2818100, BP2818500, BP28184
Nuclease-free Water (not DEPC-Treated)	MLS
(*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**.

2 Extract nucleic acid (automated method)

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

Before you begin

WARNING! Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. See the Appendix A, “Safety”.

- 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.
- Determine 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 extractions, plus 10% overage.

Sample input volume	Volume of 80% Ethanol per extraction
200 µL	0.5 mL
400 µL	1.0 mL

- Label the short side of each KingFisher™ 96 Deep-Well 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 tip comb can be used with alternate plates. See the "Required materials not supplied" on page 6 for compatible plates.

- Mark the Negative Control well on the plate.

Extract RNA (200-µL sample input volume)

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 MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit product page at **www.thermofisher.com** and loaded onto the instrument.

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

Note: During the wash steps, the 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.

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 type	Reagent	Volume per well
Wash 1 Plate	KingFisher™ 96 Deep-Well Plate	Wash Solution	500 µL
Wash 2 Plate		80% Ethanol	500 µL
Elution Plate		Elution Buffer	50 µL
Tip Comb Plate	Place a KingFisher™ 96 tip comb for DW magnets in a KingFisher™ 96 KF microplate or equivalent plate[1]		

[1] See "Required materials not supplied" on page 6 for equivalent plates.

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 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.

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

Prepare sample plate (200-µL sample input volume)

1. 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 (KingFisher™ 96 Deep-Well 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.

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. Add 5 µL of Proteinase K to each well, including the Negative Control well.

Note: Add the Proteinase K to the top layer of the solution in each well. Do not push the pipette tip into the bottom binding mix layer.

5. (*Optional*) If using an extraction control, add the required volume to each well, including the Negative Control well. For more information about using an extraction control, see the assay documentation.

Note: The Proteinase K (see step 4) and extraction control can be pre-mixed on each day of use, then kept on ice. Add the combined required volume of Proteinase K and extraction control to each well of the Sample Plate.

For example, if your assay recommends 5 µL of the extraction control per extraction, add 10 µL of pre-mixed Proteinase K and extraction control to each well during step 4.

Process the samples (200-µL sample input 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 on the KingFisher™ Flex instrument turntable in the order 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 µL of Elution Buffer (see "Prepare the processing plates (200-µL sample input volume)" on page 9).

Note:

- Significant bead carry over may adversely affect performance of RT-PCR or other downstream assays. If bead carry over is observed, place elution plate on a magnetic stand to pellet the beads, then pipette the eluate to a new 96-well plate. Review 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 or seal the plate and store at –20°C for long-term storage.

Extract RNA (400-µL sample input volume)

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 MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit product page at **www.thermofisher.com** and loaded onto the instrument.

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

Note: During the wash steps, the 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.

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 type	Reagent	Volume per well
Wash 1 Plate	KingFisher™ 96 Deep-Well Plate	Wash Solution	1,000 µL
Wash 2 Plate		80% Ethanol	1,000 µL
Elution Plate		Elution Buffer	50 µL
Tip Comb Plate	Place a KingFisher™ 96 tip comb for DW magnets in a KingFisher™ 96 KF microplate or equivalent plate[1]		

[1] See "Required materials not supplied" on page 6 for equivalent plates.

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 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.

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

Prepare sample plate (400-µL sample input volume)

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

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. Add 10 µL of Proteinase K to each well, including the Negative Control well.

   Note: Add the Proteinase K to the top layer of the solution in each well. Do not push the pipette tip into the bottom binding mix layer.

5. (*Optional*) If using an extraction control, add the required volume to each well, including the Negative Control well. For more information about using an extraction control, see the assay documentation.

   Note: The Proteinase K (see step 4) and extraction control can be pre-mixed on each day of use, then kept on ice. Add the combined required volume of Proteinase K and extraction control to each well of the Sample Plate.

   For example, if your assay recommends 10 µL of the extraction control per extraction, add 20 µL of pre-mixed Proteinase K and extraction control to each well during step 4.

Process the samples (400-µL sample input 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 on the KingFisher™ Flex instrument turntable in the order 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 µL of Elution Buffer (see “Prepare the processing plates (400-µL sample input volume)” on page 12).

Note:

- Significant bead carry over may adversely affect performance of RT-PCR or other downstream assays. If bead carry over is observed, place elution plate on a magnetic stand to pellet the beads, then pipette the eluate to a new 96-well plate. Review 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 or seal the plate and store at –20°C for long-term storage.

Extract RNA from saliva samples (200-µL sample input volume)

Guidelines for saliva collection

- Ensure that there was no eating, drinking, smoking, chewing tobacco, chewing gum, brushing teeth, or use of mouthwash for at least 30 minutes before giving a saliva sample.
- At least 30 minutes before saliva collection, rinse the mouth with water by swishing water for 10 seconds and swallowing the water to rid mouth of debris.
- Use the passive drool technique to pool saliva in the mouth, then drool into a collection device.
- Ensure only saliva is collected by using the passive drool technique, with no coughing or collection of phlegm.
- For saliva collection volume, follow the saliva collection device manufacturers instructions for use.
- For raw saliva, collect at least 1 mL.

Prepare raw saliva samples

1. Upon receipt of samples for extractions, dilute the raw saliva sample 1:1 by adding an equal volume of 1X PBS pH 7.4 (without calcium or magnesium) to the tube and vortex well at maximum speed for 1 minute.
2. Let the diluted raw saliva samples sit and settle for at least 30 minutes at 20°C to 25°C.

   Note: Gradually, 2 fractions will form. Do not disturb the layers.
3. *(Optional)* Centrifuge the diluted raw saliva sample at 1,500 x g (3,000 rpm) for 5 minutes to separate the large debris.
4. Aliquot 200 µL from the top fraction of the diluted raw saliva sample into the Sample Plate.

   Note: Pipet slowly to avoid large debris and precipitants from the lower fraction.

Prepare preserved saliva samples

1. Upon receipt of samples for extractions, let the preserved saliva samples sit and settle for at least 30 minutes at 20°C to 25°C.

   Note: In some cases, large debris may start to settle to the bottom. A clear separation may not always be visible.
2. *(Optional)* Centrifuge the preserved saliva sample at 1,500 x g (3,000 rpm) for 5 minutes to separate the large debris.
3. Aliquot 200 µL from the top fraction of the preserved saliva sample into the Sample Plate.

   Note: Pipet slowly to avoid large debris and precipitants from the lower fraction.

Before you begin (saliva samples)

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 60% Ethanol using Ethanol, Absolute, Molecular Biology Grade and Nuclease-free Water (not DEPC-Treated) for the required number of reactions, sufficient for 500 µL per reaction, plus 10% overage.
- Label the short side of each KingFisher™ 96 Deep-Well Plate (4):

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

Note: The tip comb will be placed in the Wash 2/Tip comb plate.

- Mark the Negative Control well on the plate.

Set up the instrument

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_Saliva_200_Flex_V1** program has been downloaded from **www.thermofisher.com** 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™ Clear Adhesive Film), then store at room temperature for up to 1 hour while you set up the sample plate.

Plate ID	Plate type	Reagent	Volume per well
Wash 1 Plate	KingFisher™ 96 Deep-Well Plate	Wash Solution	500 µL
Wash 2 Plate		60% Ethanol	500 µL
Elution Plate		Elution Solution	50 µL

Note: A tip comb loading plate is not necessary. The tip comb will be placed in the filled Wash 2 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	250 µL
Total Nucleic Acid Magnetic Beads	10 µL
Total volume per well	260 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)

Prepare the KingFisher™ 96 Deep-Well Plate labeled "Sample Plate".

1. Invert the Binding Bead Mix 5 times gently to mix, then add 260 µL to each sample well and the Negative Control well in the Sample Plate.

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

2. Add 200 µL of sample from the prepared saliva (see “Prepare raw saliva samples” on page 14 or “Prepare preserved saliva samples” on page 14) to the designated sample well in the Sample Plate.
3. Add 200 µL of Nuclease-free Water (not DEPC-Treated) to the Negative Control well in the Sample Plate.
4. Add 5 µL of Proteinase K into the sample layer of each well in the Sample Plate.

   Note: Add the Proteinase K to the top layer of the solution in each well. Do not push the pipette tip into the bottom binding mix layer.

Process the samples (200-µL sample input volume)

1. Add the tip comb to the filled Wash 2 plate.

   IMPORTANT! Ensure that the tip comb is added to the filled Wash 2 plate because a tip comb loading plate is not used for the protocol to extract RNA from saliva samples.

2. Select the **MVP_Saliva_200_Flex_V1** on the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head.

3. Start the run, then load the prepared plates into position on the KingFisher™ Flex instrument turntable in the order prompted by the instrument.

4. After the run is complete (~25 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 µL of Elution Solution.

Note:

- Significant bead carry over may adversely impact performance of RT-PCR or other downstream assays. If there are beads left in the elution plate after processing is complete, place the plate on a 96-well magnetic stand, collect the beads, then transfer the eluate to a new plate.
- 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, at –20°C for short-term storage, or at –80°C for long-term storage.

3 Extract nucleic acid (manual method)

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

Before you begin

WARNING! Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. See the Appendix A, “Safety”.

- 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.
- Determine the number of required extractions 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 extractions, plus 10% overage.

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

- Mark the Negative Control well on the plate.

Extract RNA (200-µL sample input volume)

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 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.

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

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

This section provides volumes for the sample plate. Your plate layout will depend on the number of samples you run.

1. 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.

2. Add 200 µL of sample to each sample well of a KingFisher™ 96 Deep-Well Plate.
3. Add 200 µL of Nuclease-free Water (not DEPC-Treated) to the Negative Control well.
4. Add 5 µL of Proteinase K to each well, including the Negative Control well.

   Note: Add the Proteinase K to the top layer of the solution in each well. Do not push the pipette tip into the bottom binding mix layer.

5. (*Optional*) If using an extraction control, add the required volume to each well, including the Negative Control well. For more information about using an extraction control, see the assay documentation.

   Note: The Proteinase K (see step 4) and extraction control can be pre-mixed on each day of use, then kept on ice. Add the combined required volume of Proteinase K and extraction control to each well of the Sample Plate.

For example, if your assay recommends 5 µL of the extraction control per extraction, add 10 µL of pre-mixed Proteinase K and extraction control to each well during step 4.

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)

Note: During the wash steps, the 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.

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 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)

1. Add 50 µL of Elution Buffer 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 performance of RT-PCR or other downstream assays. If bead carry over is observed, extend the time on the magnetic stand to further pellet the beads, then pipette the eluate to a new 96-well plate. Review assay results to determine if re-extraction is required.

Place the plate on ice for immediate use or seal plate and store at –20°C for long-term storage.

Extract RNA (400-µL sample input volume)

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 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.

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

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

This section provides volumes for the sample plate. Your plate layout will depend on the number of samples you run.

1. 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.

2. Add 400 µL of sample to each sample well of a KingFisher™ 96 Deep-Well Plate.
3. Add 400 µL of Nuclease-free Water (not DEPC-Treated) to the Negative Control well.
4. Add 10 µL of Proteinase K to each well, including the Negative Control well.

   Note: Add the Proteinase K to the top layer of the solution in each well. Do not push the pipette tip into the bottom binding mix layer.

5. (*Optional*) If using an extraction control, add the required volume to each well, including the Negative Control well. For more information about using an extraction control, see the assay documentation.

   Note: The Proteinase K (see step 4) and extraction control can be pre-mixed on each day of use, then kept on ice. Add the combined required volume of Proteinase K and extraction control to each well of the Sample Plate.
   For example, if your assay recommends 10 µL of the extraction control per extraction, add 20 µL of pre-mixed Proteinase K and extraction control to each well during step 4.

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)

Note: During the wash steps, the 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.

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 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)

1. Add 50 µL of Elution Buffer 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 affect performance of RT-PCR or other downstream assays. If bead carry over is observed, extend the time on the magnetic stand to further pellet the beads, then pipette the eluate to a new 96-well plate. Review assay results to determine if re-extraction is required.

Place the plate on ice for immediate use or seal the plate and store at –20°C for long-term storage.

Extract RNA from saliva samples (200-µL sample input volume)

Guidelines for saliva collection

- Ensure that there was no eating, drinking, smoking, chewing tobacco, chewing gum, brushing teeth, or use of mouthwash for at least 30 minutes before giving a saliva sample.
- At least 30 minutes before saliva collection, rinse the mouth with water by swishing water for 10 seconds and swallowing the water to rid mouth of debris.
- Use the passive drool technique to pool saliva in the mouth, then drool into a collection device.
- Ensure only saliva is collected by using the passive drool technique, with no coughing or collection of phlegm.
- For saliva collection volume, follow the saliva collection device manufacturers instructions for use.
- For raw saliva, collect at least 1 mL.

Prepare raw saliva samples

1. Upon receipt of samples for extractions, dilute the raw saliva sample 1:1 by adding an equal volume of 1X PBS pH 7.4 (without calcium or magnesium) to the tube and vortex well at maximum speed for 1 minute.
2. Let the diluted raw saliva samples sit and settle for at least 30 minutes at 20°C to 25°C.

   Note: Gradually, 2 fractions will form. Do not disturb the layers.
3. *(Optional)* Centrifuge the diluted raw saliva sample at 1,500 x g (3,000 rpm) for 5 minutes to separate the large debris.
4. Aliquot 200 µL from the top fraction of the diluted raw saliva sample into the Sample Plate.

   Note: Pipet slowly to avoid large debris and precipitants from the lower fraction.

Prepare preserved saliva samples

1. Upon receipt of samples for extractions, let the preserved saliva samples sit and settle for at least 30 minutes at 20°C to 25°C.

   Note: In some cases, large debris may start to settle to the bottom. A clear separation may not always be visible.
2. *(Optional)* Centrifuge the preserved saliva sample at 1,500 x g (3,000 rpm) for 5 minutes to separate the large debris.
3. Aliquot 200 µL from the top fraction of the preserved saliva sample into the Sample Plate.

   Note: Pipet slowly to avoid large debris and precipitants from the lower fraction.

Before you begin (saliva samples)

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 60% Ethanol using Ethanol, Absolute, Molecular Biology Grade and Nuclease-free Water (not DEPC-Treated) for the required number of reactions, sufficient for 500 µL per reaction, plus 10% overage.
- Mark the Negative Control well on the 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	250 µL
Total Nucleic Acid Magnetic Beads	10 µL
Total volume per well	260 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. Invert the Binding Bead Mix 5 times gently to mix, then add 260 µL to each sample well and Negative Control well in the KingFisher™ 96 Deep-Well Plate labeled 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.

2. Transfer 200 µL from the top fraction of the prepared saliva sample into the Sample Plate (see “Prepare raw saliva samples” on page 14 or “Prepare preserved saliva samples” on page 14).

   Note: Pipet slowly to avoid large debris and precipitants from the lower fraction.

3. Add 200 µL of Nuclease-free Water (not DEPC-Treated) to the Negative Control well.

4. Add 5 µL of Proteinase K to each well, including the Negative Control well.

   Note: Add the Proteinase K to the top layer of the solution in each well. Do not push the pipette tip into the bottom binding mix layer.

5. (*Optional*) If using an extraction control, add the required volume to each well, including the Negative Control well. For more information about using an extraction control, see the assay documentation.

   Note: The Proteinase K (see step 4) and extraction control can be pre-mixed on each day of use, then kept on ice. Add the combined required volume of Proteinase K and extraction control to each well of the Sample Plate.
   For example, if your assay recommends 5 µL of the extraction control per extraction, add 10 µL of pre-mixed Proteinase K and extraction control to each well during step 4.

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 10 minutes (ensure the bottom of the plate is uncovered), then shake the plate at 1,050 rpm for 2 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. 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 60% Ethanol.

8. Repeat step 2 to step 6 using 250 µL of 60% Ethanol.

9. If there is any 60% Ethanol 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 sample input volume)

1. 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 2 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 2 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 performance of RT-PCR or other downstream assays. If there are beads left in the elution plate after processing is complete, place the plate on a 96-well magnetic stand, collect the beads, then transfer the eluate to a new plate. Review assay results to determine if re-extraction is required.

Place the Elution Plate on ice for immediate use, at –20°C for short-term storage, or at –80°C for long-term storage.

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, see the “Documentation and Support” section in this document.

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.

- 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
- 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

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

B

Documentation and support

Related documentation

Document	Publication Number
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**.