Procedure for viral nucleic acid isolation from 200 µL of saliva sample

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product information

This user bulletin describes the procedure to isolate viral nucleic acid from 200 µL of saliva samples (raw or preserved) with the following kits:

- MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Cat. No. A42352, Cat. No. A48310)
- MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit (Cat. No. A48383R)

The procedure for automated extractions performed with the KingFisher™ Flex Purification System (KingFisher) are described.

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™ 96 Deep-Well Plate	95040450 A48305 A48424 95040455
KingFisher™ 96 tip comb for DW magnets	97002534 A48438 A48414
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
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
PBS (1X), pH 7.4 (without calcium and magnesium)	10010023
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.

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

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.

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

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 (Cat. No. A42352, Cat. No. A48310) or MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit (Cat. No. A48383R) with a sample input volume of 200 µL.

1 Set up the instrument (200-µL sample input volume)

1.1. 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.

1.2. Ensure that the **MVP_Saliva_200_Flex_V1** program has been downloaded from the instrument product page at **www.thermofisher.com** and loaded onto the instrument.

2 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 20°C to 25°C 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™ 96 Deep-Well Plate	Wash Solution	500 µL
Wash 2/Tip comb plate	3		60% Ethanol	500 µL
Elution Plate	4		Elution Solution	50 µL

Note: Ensure to use 60% ethanol in Wash 2 plate.

Note: Place the tip comb in Wash 2/Tip comb plate.

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

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

3.1. Vortex the Total Nucleic Acid Magnetic Beads to ensure that the bead mixture is homogeneous.

3.2. 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.

3.3. Mix well by inversion, then store at 20°C to 25°C.

Note: Do not vortex or quick spin the Master Mix to avoid bubbles.

4 Prepare sample plate (200-µL sample input volume)

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

4.1. 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.

4.2. Add 200 µL of sample from the prepared saliva (see “Prepare raw saliva samples” on page 2 or “Prepare preserved saliva samples” on page 2) to the designated sample well in the Sample Plate.

4.3. Add 200 µL of Nuclease-free Water (not DEPC-Treated) to the Negative Control well in the Sample Plate.

4.4. Add 5 µL of Proteinase K into the sample layer of each well in the Sample Plate.

Note: Do not pipet directly down into the Binding Bead Mix layer.

5 Process the samples (200-µL sample input volume)

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

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

5.3. 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.

Note: Significant bead carry over may adversely impact RT-PCR performance. If there are beads left over in the elution plate, place the plate on a magnetic stand and transfer the eluate to a clean plate.

Place the Elution Plate on ice for immediate use in real-time RT-PCR, or at -20°C for short term storage, or at -80°C for long term storage.

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**.

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

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Revision	Date	Description
C.0	08 February 2021	Added the Cat. Nos. after the kit names to clarify which kits to use.
B.0	09 November 2020	Removed the following text from the document: ViewRNA Cell Assay Kit.
A.0	14 October 2020	New document.