KingFisher™ Ready MagMAX™ Viral/Pathogen II Prefilled Plates

Isolation of viral nucleic acid from 200 µL of transport media or saliva samples (raw or preserved) using prefilled single-use kits for automation

Catalog Number A47183

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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 description

The Thermo Scientific™ KingFisher™ Ready MagMAX™ Viral/Pathogen II Prefilled Plates (Cat. No. A47183) is specifically designed for scalable, rapid purification of high-quality total nucleic acid (RNA and DNA) from easy-to-lyse virus. 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 protocol guides users through automated isolation of RNA and DNA from raw and stabilized saliva samples using the KingFisher™ Flex Magnetic Particle Processor. The kit is also compatible with transport media specimens without any alterations to the workflow.

Contents and storage

Reagents that are provided in each kit support 96 reactions.

IMPORTANT! On receipt, store all plates and reagents in an upright position at room temperature (15°C to 25°C).

Table 1 KingFisher™ Ready MagMAX™ Viral/Pathogen II Prefilled Plates

Component	Quantity	Storage
96 deep well plate filled with Binding Solution. Samples will be added to this plate.	1	Store upright at 15°C to 25°
96 deep well plate filled with Magnetic Beads	1	
96 deep well plate filled with Wash I Solution	1	
96 deep well plate filled with Wash II Solution	1	
Empty, sealed 96 deep well plate to be filled with Elution Solution	1	
96 deep well tip comb nested in a 96 deep well plate	1	
Elution Solution	1 bottle	
Proteinase K Solution	1 bottle	

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.

Table 2 For automated protocol

Item	Source
Instrument	
KingFisher™ Flex Magnetic Particle Processor 96DW with 96 Deep-well Head	5400630
KingFisher™ Flex 96 Deep-Well Heating Block (do not use a standard heating block)	24075430
Equipment	
Single and multichannel adjustable pipettors (1 µL to 1,000 µL)	MLS
Laboratory mixer, vortex, or equivalent	MLS
Cold block or ice	MLS
Tubes, plates and other consumables	
PBS (1x), pH 7.4 (without calcium and magnesium)	10010023
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Adhesive Film Applicator	4333183
Nonstick, RNase-free microcentrifuge tubes (1.5 mL and 2.0 mL)[1]	thermofisher.com/plastics
Sterile aerosol barrier (filtered) pipette tips[1]	thermofisher.com/pipettetips

[1] Available at fisherscientific.com

Procedural guidelines

- The plates provided in this kit are single use plates only. Do not reuse the plates.
- Ensure plates are stored upright for 24 hours before opening.
- Perform all steps at room temperature (15–25°C) unless otherwise noted.
- Yellowing of the Lysis/Binding and Wash I Solution is normal and will not impact buffer performance.

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.

Before each use of the kit

- Gently mix reagents in bottles before use. Avoid creating bubbles.

- Examine the plate containing the beads prior to opening. Ensure that the beads are at the bottom of each well. Hold the plate by the narrow end and sharply swing the sealed plate in a downward motion 5 times. Hold the plate on the opposite side and repeat. The plate may be gently centrifuged at 2,100 x *g* for 10-20 seconds if needed.

  IMPORTANT! Do not over centrifuge the bead plate. This will compact the bead pellet and make it difficult for the instrument to pick up the beads from the plate.

  Note: Beads are provided in excess in the bead plate. Some beads or coloration may remain in the bead plate after the extraction process has completed. This will not impact overall performance.

- To remove seals from prefilled plates, place the prefilled plate squarely onto the benchtop, secure the plate with one hand and grasp the seal at the lower left corner with the other hand. Using a gentle but steady motion, peel the seal off of the plate diagonally toward the upper right corner without jostling the contents.

- If required by your assay, designate one well of the Sample Plate for a negative extraction control.

Prepare saliva samples for extraction

Prepare raw saliva samples

1. Upon receipt of samples for extraction, 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. Transfer 200 µL from the top fraction of the diluted raw saliva sample to 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 extraction, 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. Transfer 200 µL from the top fraction of the preserved saliva sample to the Sample Plate.

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

Perform Viral Nucleic Acid Isolation from 200 µL of transport media or 200 µL of prepared saliva samples using the KingFisher™ Flex instrument

IMPORTANT! Do not attempt to process more than the maximum volume allowed for each sample type. Yields and quality will be reduced.

1 Set up the instrument

1.1. Ensure that the instrument is set up with the proper magnetic head and the proper heat block, as indicated in the following table.

Component	Type
Magnetic head	96 deep well magnetic head
Heat block	96 deep well heat block

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

1.2. Ensure that the proper program (**MVPII_Ready_Saliva_200_Flex_v1** or **MVPII_Ready_TransportMedia_200_Flex_v1**) has been loaded onto the instrument.

2 Prepare Sample Plate and Elution Plate

2.1. Remove the Elution Plate and the bottle of Elution Solution from the product box.

2.2. Remove the seal from the empty Elution Plate (for instructions on removing plate seals, see “Before each use of the kit” on page 2).

This is the final Elution Plate which will be slotted into deck position 5 on the instrument after the Elution Solution is added.

2.3. Add 50 µL of the Elution Solution to each well of the empty Elution Plate. Cover the Elution Plate with a temporary seal to prevent contamination.

2.4. Remove the Sample Plate and the bottle of Proteinase K from the box.

2.5. Remove the seal from the Sample Plate (for instructions on removing plate seals, see “Before each use of the kit” on page 2).

This is the Sample Plate which will be slotted into deck position 2 on the instrument after the samples, Proteinase K, and negative control are added.

2.6. Add 200 µL of sample to the designated well in the Sample Plate.

2.7. Add the following reagents to the Sample Plate.

- Nuclease-free water (not DEPC-Treated): Add 200 µL to the negative control well.
- Proteinase K: Add 5 µL to the sample layer of each sample-containing well.

Note: Do not push the pipette tip into the bottom binding mix layer.

- *(Optional)* Extraction control: Add the appropriate amount as described in the user guide for the kit.

2.8. Remove the tip comb and the remaining sealed reagent plates from the product box.

2.9. Remove the seals from the reagent plates (for instructions on removing plate seals, see “Before each use of the kit” on page 2).

2 Prepare Sample Plate and Elution Plate *(continued)*

2.10. Confirm KingFisher™ Flex deck positions with the following table, then start the run.

Note: Deck positions are also denoted on labels on the short and long ends of the plate.

Deck position	Prefilled plate	Contents	Prefilled volume
1	Bead Plate	Magnetic beads + water	400 µL
2	Sample Plate	Binding Solution + Proteinase K (+extraction control if needed) + sample	250 µL
3	Wash I	Wash I	500 µL
4	Wash II	Wash II (60% EtOH)	500 µL
5	Elution Plate	Elution Solution	50 µL[1]
6	Tip comb	Tip comb	96 deep well tip comp nested in a 96 deep well plate

[1] Elution Solution is not prefilled and must be added before plate is placed on the instrument for sample extraction.

2.11. At the end of the run, immediately remove the Elution Plate from the instrument, place on ice and cover with MicroAmp™ Clear Adhesive Film, then transfer the eluate to the final tube/plate of choice for final storage.

IMPORTANT! Immediately seal the plate containing the eluate to prevent evaporation.

Note: Significant bead carryover in the eluate 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 instrument, perform preventive maintainance as instructed by the manufacturer.

Troubleshooting

Observation	Possible cause	Recommended action
Low or inconsistent yield	Plates were stored incorrectly.	Store plates in an upright position at room temperature. Examine the plate or row containing the beads before removing the seal for an indication of how to proceed.
		For plates that have been inverted, store them upright for at least 24 hours, then check that the beads form a tight dark pellet in the center of the bottom of the well before unsealing the plate.
		For plates without beads in that were not stored correctly, flick the plates in a fast downward motion to ensure that the materials are in the well and not on the seal before unsealing.
		For plates that were stored inverted, the beads are dry and uneven in the bottom of the wells and or not fully resuspended. Flick the plate in a fast downward motion to remove the fluid from the seal, then gently vortex to resuspend the beads before unsealing. A gentle brief centrifugation can be performed after resuspension but is not always necessary.
	After removing the seal, there were some wet or dry beads on the seal.	For wet beads, carefully pipet the liquid from the seal back to its proper well.
		For dry beads, resuspend the dry beads in Nuclease-Free water, then carefully pipet the liquid from the seal back to its proper well.
	There were bubbles in the wells.	Centrifuge the plates to remove the bubbles before use.
	The wells were blocked.	Remove any seal covering well openings and blocking tip comb access. Seal remnants on the plate edge will not interfere with tip comb access and do not have to be removed.
		If the seal has delaminated and left a transparent seal over the well then rotate plate 180 degrees and peel diagonally from the corner that is now on the bottom left. If the problem persists, call technical support.
	Heat block was installed incorrectly.	Install the correct deep well heat block.
	Sample input limits were below or above recommended amounts.	Consult user guide for recommended sample input ranges.

Limited product warranty

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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.

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Revision	Date	Description
B.0	14 December 2021	The manual was updated to include transport media.
A.0	7 May 2021	New manual for KingFisher™ Ready MagMAX™ Viral/Pathogen II Prefilled Plates.

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