applied biosystems USER GUIDE

MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit

High throughput isolation of viral and pathogen nucleic acid (RNA and DNA) from biofluids and transport media

Catalog Number A42356

Pub. No. MAN0018075 **Rev.** B.0

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 Applied Biosystems™ MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit is developed for scalable, rapid purification of high-quality nucleic acid (RNA and DNA) from virus, bacteria, and yeast/fungi in biofluid and transport media samples. You can use the nucleic acid purified with this kit in a broad range of molecular biology downstream applications, such as sequencing and qPCR. This protocol guides users through automated isolation of nucleic acid using the KingFisher™ Flex and the KingFisher™ Duo Prime instruments.

Contents and storage

Reagents that are provided in the kit are sufficient for 100 reactions with standard volume input or 20 reactions with large volume input.

Table 1 Components of MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit (Cat. No. A42356)

Component	Amount	Storage
Binding Solution	53 mL	15°C to 25°C
Wash Buffer	100 mL	
Elution Solution	10 mL	
Proteinase K	1 mL	
Total Nucleic Acid Binding Beads	2 mL	
Enzyme Mix	5 mL	–15°C to 25°C

For 1,000 reaction volume, use Cat. No. A42359 (Binding Solution), A42360 (Wash Buffer), A42364 (Elution Solution), A42363 (Proteinase K), A42362 (Binding Beads), and A42366 (Enzyme Mix).

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Instrument	
Magnetic particle processor (one of the following, depending on quantity/volume of sample to be processed):	
For standard volume sample[1]: KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head	5400630
For large volume sample[2]: KingFisher™ Flex Magnetic Particle Processor with 24 Deep-Well Head	5400640
KingFisher™ Duo Prime Magnetic Particle Processor	5400110
Consumables	
Deep-well plates:	
For standard volume sample[1]: KingFisher™ deep-well 96 plate	95040450
For large volume sample[2]: KingFisher™ Flex 24 deep-well plate	95040470
96-well standard plates (for use with KingFisher™ Flex only; tip comb placement and eluate storage):	
KingFisher™ 96 KF plate	97002540
Tip comb, compatible with the magnetic particle processor used:	
KingFisher™ Duo Prime 12-tip comb, for use with KingFisher™ deep-well 96 plate	97003500
KingFisher™ Duo Prime 6-tip comb, for use with KingFisher™ Flex 24 deep-well plate	97003510
KingFisher™ 96 tip comb for deep-well magnets, KingFisher™ Flex protocol only	97002534
KingFisher™ Flex 24 deep-well tip comb and plate, KingFisher™ Flex protocol only	97002610
Elution strip (for use with KingFisher™ Duo Prime only; elution step):	
KingFisher Duo elution strip	97003520
KingFisher Duo cap for elution strip	97003540

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

Item	Source
Equipment	
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Materials	
MicroAmp™ Clear Adhesive Film	4306311
Conical Tubes (15 mL)	AM12500
Conical Tubes (50 mL)	AM12501
Reagent reservoirs	MLS
Nonstick, RNase-Free Microfuge Tubes, 1.5 mL	AM12450
Nonstick, RNase-Free Microfuge Tubes, 2.0 mL	AM12475
Reagents	
Ethanol, 100% (molecular biology grade)	MLS
Nuclease-free Water	AM9932
PBS (1X), pH 7.4	10010001

[1] Standard volume sample is 200–400 µL.
[2] Large volume sample is 500 µL–2 mL.

General guidelines

- Perform all steps at room temperature (20–25°C), unless otherwise noted.
- Precipitates can occur if the Binding Solution is stored when room temperature is too cold. If there are precipitates, warm the Binding Solution at 37°C and gently mix to dissolve the precipitates. Avoid creating bubbles.
- Reagent Mix tables are sufficient for a single reaction. To calculate volumes for other sample numbers, see the per-well volume and add at least 10% overage.

Guidelines for Binding Bead Mix

- Vortex Binding Beads thoroughly before each use.
- Ensure that the beads stay fully mixed within the solution during pipetting.
- Avoid creating bubbles during mixing and aliquoting.
- Binding/Bead Mix is very viscous so pipet with care to ensure that the correct volume is added to the sample.

Before first use of the kit

- Prepare 80% Ethanol from 100% absolute Ethanol and Nuclease-Free Water.
  - For standard volume input: Prepare enough for 1.5 mL per reaction.
  - For large volume input: Prepare enough for 6 mL per reaction.

(Optional) Before each use of the kit with standard volume of vaginal microbiota and urinary tract samples

1. Add 1 mL of sample per well of a 96 deep-well plate.
2. Cover the plate, then centrifuge for 15 minutes at 2,250 × *g*.
3. Remove as much of the supernatant as possible, then add 200 µL of 1X PBS to each sample.
4. Proceed to the nucleic acid purification according to the following protocols.
   - “Perform total nucleic acid purification using KingFisher™ Flex (standard volume: 200–400 µL)“ on page 2
   - “Perform total nucleic acid purification using KingFisher™ Duo Prime (standard volume: 200–400 µL) “ on page 6

Perform total nucleic acid purification using KingFisher™ Flex (standard volume: 200–400 µL)

1 Set up the instrument

a. 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 well deep-well heat block

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

b. Ensure that the proper program (**MVP_Ultra_Flex**) has been downloaded from the product page and loaded onto the instrument.

2 Set up the processing plates

Set up the Wash, Elution, and Tip Comb Plates outside the instrument according to the following table.

Plate ID	Plate position	Plate type	Reagent	Volume per well
Wash 1 Plate	2	Deep-well	Wash Buffer	1,000 µL
Wash 2 Plate	3	Deep-well	80% Ethanol	1,000 µL
Wash 3 Plate	4	Deep-well	80% Ethanol	500 µL
Elution Plate	5	Deep-well	Elution Solution	60–100 µL
Tip Comb	6	Place a 96 Deep-well Tip Comb in a Standard Plate		

3 Digest with Enzyme Mix

a. Gently swirl Enzyme Mix, then add 50 µL per sample well in a Deep-well 96-well plate.

This is the Sample Plate.

b. Add 200–400 µL of sample to wells with Enzyme Mix.

Note: Recommend up to 200 µL input for whole blood.

c. Select the program **MVP_Ultra_Flex** on the instrument.

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

4 Prepare Binding Bead Mix

a. Vortex Beads vigorously to ensure they are homogenous.

b. Prepare Binding Bead Mix according to the following table and sample input volume:

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

[1] Use 10% Overage calculation when making a master mix for use with multiple samples.

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

5 Digest with Proteinase K, then elute nucleic acid

a. When prompted (~20 minutes after start of protocol), remove Sample Plate from instrument.

b. Add 10 µL of Proteinase K to each sample in the Sample Plate.

c. Invert Binding Bead Mix gently to mix, then add 550 µL to each sample 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 mixture containing the Binding Beads is viscous. Therefore, pipet slowly to ensure that the correct amount is added. DO NOT use a repeat pipet to add to the samples as the high viscosity will cause variations in volume added.

d. Load the Sample Plate back onto the instrument, then press **Start**.

e. After the protocol is complete (~30 minutes after adding Binding Bead Mix), immediately remove the elution plate from the instrument and cover the plate or transfer the eluate to a tube or plate of choice for final storage.

The purified nucleic acid is ready for immediate use. Alternatively, store the plate at –20°C for long-term storage.

Perform total nucleic acid purification using KingFisher™ Flex (large volume: 500 µL to 2 mL)

1 Set up the instrument

a. 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	24 deep-well magnetic head
Heat block	24 well heat block

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

b. Ensure that the proper program (**MVP_Ultra_Flex_LV**) has been downloaded from the product page and loaded onto the instrument.

2 Set up the processing plates

Set up the Wash, Elution, and Tip Comb Plates outside the instrument according to the following table.

Plate ID	Plate position	Plate type	Reagent	Volume per well
500 µL sample input				
Wash 1 Plate	2	24 Deep-well	Wash Buffer	2,000 µL
Wash 2 Plate	3	24 Deep-well	80% Ethanol	2,000 µL
Wash 3 Plate	4	24 Deep-well	80% Ethanol	2,000 µL
Elution Plate	5	24 Deep-well	Elution Solution	150 µL
Tip Comb	6	Place a 24 Deep-well Tip Comb in a Standard Plate		
>500 µL – 1 mL sample input				
Wash 1 Plate	2	24 Deep-well	Wash Buffer	4,000 µL
Wash 2 Plate	3	24 Deep-well	80% Ethanol	4,000 µL
Wash 3 Plate	4	24 Deep-well	80% Ethanol	2,000 µL
Elution Plate	5	24 Deep-well	Elution Solution	200 µL
Tip Comb	6	Place a 24 Deep-well Tip Comb in a Standard Plate		
>1 mL – 2 mL sample input				
Wash 1 Plate	2	24 Deep-well	Wash Buffer	4,000 µL
Wash 2 Plate	3	24 Deep-well	80% Ethanol	4,000 µL
Wash 3 Plate	4	24 Deep-well	80% Ethanol	2,000 µL
Elution Plate	5	24 Deep-well	Elution Solution	250 µL
Tip Comb	6	Place a 24 Deep-well Tip Comb in a Standard Plate		

3 Digest with Enzyme Mix

a. Gently swirl the Enzyme Mix, then add the appropriate volume per sample well in a deep-well 96-well plate according to the following table:

For sample input volume	Add Enzyme Mix
500 µL	100 µL
>500 µL – 2 mL	200 µL

b. Add 500 µL to 2 mL of sample to wells with Enzyme Mix.

Note: Recommend up to 1 mL input for whole blood.

3 Digest with Enzyme Mix *(continued)*

c. Select the program **MVP_Ultra_Flex_LV** on the instrument.

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

4 Prepare Binding Bead Mix

a. Vortex Beads vigorously to ensure they are homogenous.

b. Prepare Binding Bead Mix according to the following table and sample input volume:

Component	Volume per well[1]
500 µL sample input	
Binding Solution	2,700 µL
Total Nucleic Acid Magnetic Beads	50 µL
Total volume	**2,750 µL**
500 µL – 2 mL sample input	
Binding Solution	2,700 µL
Total Nucleic Acid Magnetic Beads	100 µL
Total volume	**2,800 µL**

[1] Use 10% Overage calculation when making a master mix for use with multiple samples.

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

5 Digest with Proteinase K, then elute nucleic acid

a. When instructed by the instrument (~20 minutes after the start of the protocol), remove the Sample Plate, then add Proteinase K to each sample according to the following table:

For Sample input volume	Add Proteinase K
500 µL	25 µL
>500 µL – 2 mL	50 µL

b. Invert the Binding Bead Mix gently to mix, then add Binding Bead Mix to each sample according to the following table:

For sample input volume	Add Binding Bead Mix
500 µL	2,750 µL
>500 µL – 2 mL	2,800 µL

Note: Remix Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples/wells. Mixture is viscous, pipet slowly to ensure that the correct amount is added. DO NOT use a repeat pipet to add to the samples as the high viscosity will cause variations in volume added.

c. Immediately place the plate back onto the instrument, then follow the prompts on the instrument to allow the sample processing to proceed.

d. At the end of the run (~45 minutes after adding the Binding Bead Mix), immediately remove the elution plate from the instrument and cover the plate or transfer the eluate to a tube or plate of choice for final storage.

The purified nucleic acid is ready for immediate use. Alternatively, store the plate at –20°C for long-term storage.

Perform total nucleic acid purification using KingFisher™ Duo Prime (standard volume: 200–400 µL)

1 Set up the instrument

a. 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	12-tip magnetic head
Heat block	12 well heat strip

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

b. Ensure that the proper program (**MVP_Ultra_Duo**) has been downloaded from the product page and loaded onto the instrument.

2 Set up the Sample Plate and Elution Strip

Set up the Sample Plate and Elution Strip according to the following tables, respectively.

Table 2 Sample plate

Row ID	Plate Row	Reagent	Volume per well
Sample	A	Sample	Varies
—	B	Empty	
Wash 1	C	Wash Buffer	1,000 µL
—	D	Empty	
Wash 2	E	80% Ethanol	1,000 µL
—	F	Empty	
Wash 3	G	80% Ethanol	500 µL
Tip Comb	H	Tip Comb	

Table 3 Elution strip

Row ID	Plate Row	Reagent	Volume per well
Elution	A	Elution Solution	60–100 µL

3 Digest with Enzyme Mix

a. Gently swirl Enzyme Mix, then add 50 µL per sample well in Row A.

b. Add 200–400 µL of sample to wells with Enzyme Mix.

Note: Recommend up to 200 µL input for whole blood.

c. Select the program **MVP_Ultra_Duo** on the instrument.

d. Start the run, then load the Elution Strip and Sample Plate into position when prompted by the instrument.

4 **Prepare Binding Bead Mix**

a. Vortex Beads vigorously to ensure they are homogenous.

b. Prepare Binding Bead Mix according to the following table and sample input volume:

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

[1] Use 10% Overage calculation when making a master mix for use with multiple samples.

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

5 **Digest with Proteinase K, then elute nucleic acid**

a. When prompted (~20 minutes after start of protocol), remove Sample Plate from instrument.

b. Add 10 µL of Proteinase K to each sample in Row A of the Sample Plate.

c. Invert Binding Bead Mix gently to mix, then add 550 µL to each sample in Row A of 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 mixture containing the Binding Beads is viscous. Therefore, pipet slowly to ensure that the correct amount is added. DO NOT use a repeat pipet to add to the samples as the high viscosity will cause variations in volume added.

d. Load the Sample Plate back onto the instrument, then press **Start**.

e. After the protocol is complete (~30 minutes after adding Binding Bead Mix), immediately remove the Elution strip from the instrument. Cover with the Elution Strip Cap for temporary storage, or transfer the eluate to a tube or plate of choice for final storage.

The purified nucleic acid is ready for immediate use. Alternatively, store the plate at –20°C for long-term storage.

Perform total nucleic acid purification using KingFisher™ Duo Prime (large volume: 500 µL – 2 mL)

1 **Set up the instrument**

a. 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	6-tip magnetic head
Heat block	Both 6 well heat strips

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

b. Ensure that the proper program (**MVP_Ultra_Duo_LV**) has been downloaded from the product page and loaded onto the instrument.

2 Set up the processing plates

Set up the Sample Plate and Elution Plate according to the following tables and sample input volume.

Table 4 24 deep-well plate layout (500 µL sample input)

Row ID	Plate Row	Reagent	Volume per well
Sample plate			
Sample	A	Sample	Varies
Wash 1	B	Wash Buffer	2,000 µL
Wash 2	C	80% Ethanol	2,000 µL
Wash 3	D	80% Ethanol	2,000 µL
Elution plate			
Elution	A	Elution Solution	150 µL
Tip Comb	B	6-Tip Comb in a 24 Deep-well plate	

Table 5 24 deep-well plate layout (>500 µL – 1 mL sample input)

Row ID	Plate Row	Reagent	Volume per well
Sample plate			
Sample	A	Sample	Varies
Wash 1	B	Wash Buffer	4,000 µL
Wash 2	C	80% Ethanol	4,000 µL
Wash 3	D	80% Ethanol	2,000 µL
Elution plate			
Elution	A	Elution Solution	200 µL
Tip Comb	B	6-Tip Comb in a 24 Deep-well plate	

Table 6 24 deep-well plate layout (>1 mL – 2 mL sample input)

Row ID	Plate Row	Reagent	Volume per well
Sample plate			
Sample	A	Sample	Varies
Wash 1	B	Wash Buffer	4,000 µL
Wash 2	C	80% Ethanol	4,000 µL
Wash 3	D	80% Ethanol	2,000 µL
Elution plate			
Elution	A	Elution Solution	250 µL
Tip Comb	B	6-Tip Comb in a 24 Deep-well plate	

3 Digest with Enzyme Mix

a. Gently swirl the Enzyme Mix, then add the appropriate volume per sample well in Row A of the Sample Plate according to the following table:

For sample input volume	Add Enzyme Mix
500 µL	100 µL
>500 µL – 2 mL	200 µL

b. Add 500 µL to 2 mL of sample to wells with Enzyme Mix.

Note: Recommend up to 1 mL input for whole blood.

3 Digest with Enzyme Mix *(continued)*

c. Select the program **MVP_Ultra_Duo_LV** on the instrument.

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

4 Prepare Binding Bead Mix

a. Vortex Beads vigorously to ensure they are homogenous.

b. Prepare Binding Bead Mix according to the following table and sample input volume:

Component	Volume per well[1]
500 µL sample input	
Binding Solution	2,700 µL
Total Nucleic Acid Magnetic Beads	50 µL
Total volume	**2,750 µL**
500 µL – 2 mL sample input	
Binding Solution	2,700 µL
Total Nucleic Acid Magnetic Beads	100 µL
Total volume	**2,800 µL**

[1] Use 10% Overage calculation when making a master mix for use with multiple samples.

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

5 Digest with Proteinase K, then elute nucleic acid

a. When instructed by the instrument (~20 minutes after the start of the protocol), remove the Sample Plate, then add Proteinase K to each sample according to the following table:

For Sample input volume	Add Proteinase K
500 µL	25 µL
>500 µL – 2 mL	50 µL

b. Invert the Binding Bead Mix gently to mix, then add Binding Bead Mix to each sample in Row A of the Sample Plate according to the following table:

For sample input volume	Add Binding Bead Mix
500 µL	2,750 µL
>500 µL – 2 mL	2,800 µL

Note: Remix Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples/wells. Mixture is viscous, pipet slowly to ensure that the correct amount is added. DO NOT use a repeat pipet to add to the samples as the high viscosity will cause variations in volume added.

c. Immediately place the plate back onto the instrument, then follow the prompts on the instrument to allow the sample processing to proceed.

d. At the end of the run (~45 minutes after adding the Binding Bead Mix), immediately remove the Elution Plate from the instrument and cover the plate or transfer the eluate to a tube or plate of choice for final storage.

The purified nucleic acid is ready for immediate use. Alternatively, store the plate at –20°C for long-term storage.

Limited product warranty

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Revision	Date	Description
B.0	06 December 2019	Updated Total Nucleic Acid Binding Buffer to Binding Solution.
A.0	15 March 2019	New document.

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